American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Agents: For the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Australia: Angus and Robertson, Limited, 89–95 Castlereagh Street, Sydney; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 DECEMBER 1949 NUMBER 12

CONTENTS

The Pathology of Chemical Carcinogenesis. H. L. STEWART 1095

Solitary "Tumors" of Atypical Amyloid (Paramyloid). W. H. BAUER AND J. F. KUZMA. 1097

An Improved Method of Counting Blood Platelets. And Its Evaluation in Blood of Rabbits. G. J. SCHEFF AND P. H. RALPH 1113

Effect of Splenic Extracts on Blood Platelet Counts In Rabbits. G. J. SCHEFF AND I. M. SCHEFF 1122

Replacement Transfusion Following Administration of Incompatible Blood. Report of Case with Successful Outcome. J. E. CONLEY, J. ERBES, F. J. STODDARD AND J. W. FRYE.. 1131

Behavior of Virus of Poliomyelitis in a Culture of *Endamoeba histolytica*. V. M. YOUNG, O. FELSENFELD AND C. L. BYRD 1135

Congenital Pulmonary Aspiration-Tubeculosis. Report of a Case. A. J. HERTZOG, S. CHAPMAN, AND J. HERRING 1139

Fulminating Septicemia Caused by *Mima polymorpha*. Report of Case. J. FAUST AND M. HOOD 1143

Clinicopathologic Conference. II. POPPER AND I. F. VOLINI 1146

Editorials:

Neuropathology. J. W. KERNOHAN 1154

What is the Trend? O. A. BRINES 1155

On Shortening Scientific Papers 1155

Book Reviews 1156

Selected Abstracts 1161

Obituaries 1166

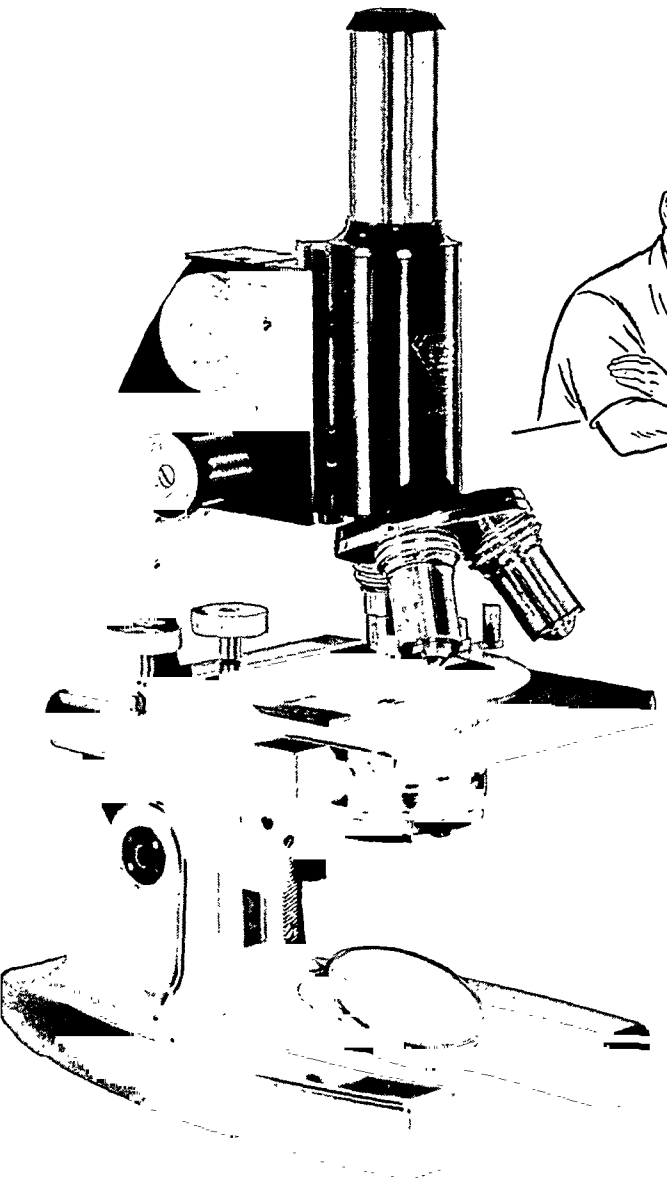
Index of Subjects, Volume 19 1169

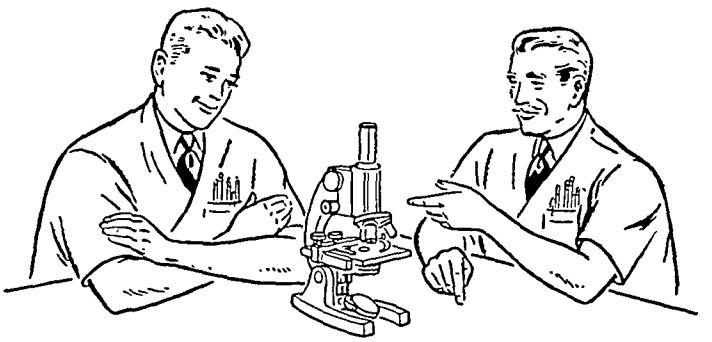
Index of Authors, Volume 19 1183

Table of Contents, Volume 19 iii

(In writing to advertisers, please mention the Journal—it helps.)

Years Ahead IN SERVICE-PROVED ADVANTAGES





● You can be sure that all performance advantages of Bausch & Lomb Laboratory Microscopes have been completely *service-proved* in the field.

Some of these time-tested advantages are still exclusive with Bausch & Lomb. Other mechanical and optical advancements, later adopted by the field generally, will be recognized by experienced microscopists as features *originated, developed,* and *service-proved* in past years by Bausch & Lomb. The "bugs" are out . . . every advancement on Bausch & Lomb Laboratory Microscopes has been brought to its maximum operating efficiency, accuracy and durability.

For service-proved performance that is years ahead . . . be sure your new laboratory microscope is a "Bausch & Lomb."

WRITE for complete information and demonstration to Bausch & Lomb Optical Co., 652-L St. Paul St., Rochester 2, N.Y.

Bausch & Lomb *Laboratory* Microscopes

we say they're better

and so will you when you've

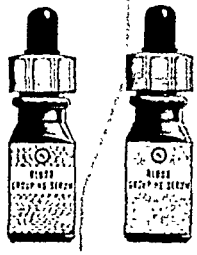
proved it in your own laboratory

HYLAND BLOOD GROUPING SERUM

Anti-A and Anti-B

Prepared from carefully selected donors to assure a highly potent, rapid acting, and specific product. Accurate results with maximum speed.

Available in 2 cc. and 5 cc. sets.



HYLAND ANTI-Rh SERUMS

Anti-Rh_0 (Anti-D) Typing Serum

Anti-Rh_0 rh' rh" (Anti-CDE) Typing Serum

Outstanding serum for slide method—rigidly tested for titer, avidity, specificity and sterility. Prepared from selected, immunized donors.

Both types available in 2 cc. and 5 cc. sizes.



make this test at our expense!

Clip the coupon for your free samples today! You'll receive generous samples of Rh_0 Typing Serum and Blood Grouping Serum. Enough to convince you that their speed, accuracy and titer are unsurpassed.

Hyland Laboratories

4534 Sunset Boulevard, Los Angeles 27, California

Please send me generous samples of Rh_0 and Blood Grouping Serums.

Your Name_____

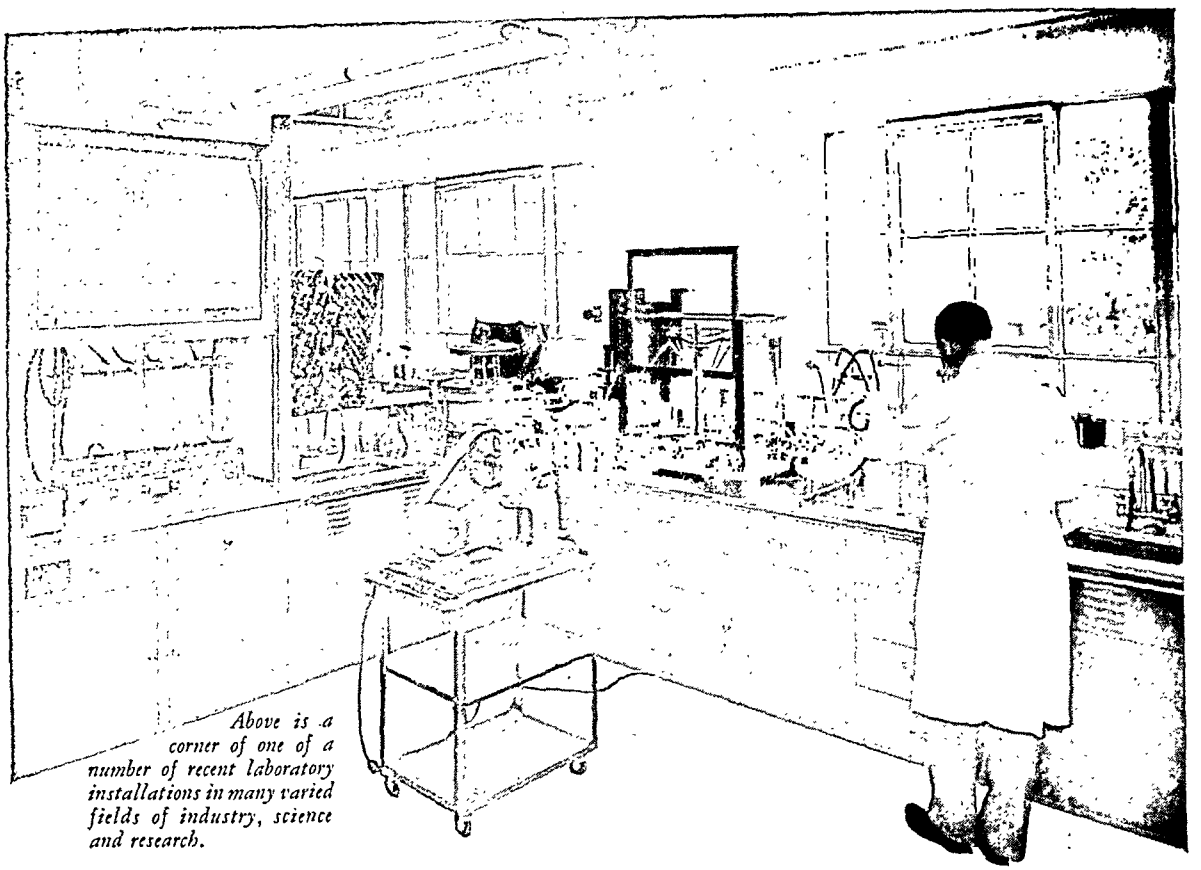
Hospital or Lab. Name_____

Address_____

City and State_____

From what dealer do you regularly buy your typing serums?_____

(In writing to advertisers, please mention the Journal—it helps.)



Above is a corner of one of a number of recent laboratory installations in many varied fields of industry, science and research.

you can save by using a. s. aloe

as your complete source for laboratory supplies

Whether you are expanding, buying replacement equipment, or planning a new laboratory, one complete source for your laboratory supplies saves you *dollars* through lower prices on quantity purchases . . . *time* in placing numerous orders . . . *manpower* in handling multiple shipments.

Furniture, Apparatus, Chemicals

Our complete stocks are geared to all your laboratory needs. Aloe's new stainless steel Moduline Furniture has already proved exceptionally satisfactory for the modern laboratory. Built in standard units, this furniture incorporates the savings of mass production with maximum versatility for long range planning.

Our apparatus stocks include a wide selection of items for routine and special work. Inorganic and organic chemicals, rare sugars, culture media, dyes and stains are always in stock.

Service Departments

We maintain a Planning Department for working up plans on new installations and a Research Laboratory to help locate unusual items or procedures, and in preparing special reagents. Both at your service without charge.

Complete Catalogs

If you do not have copies on file of our General Laboratory Apparatus and Equipment Catalog No. 102 and Moduline Furniture Bulletin No. T-300, send for them today.

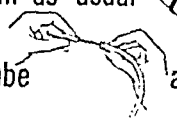
a. s. aloe company *Serving Science Since 1860*

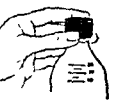
1831 Olive Street • St. Louis 3, Missouri

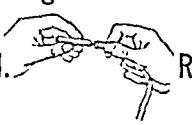
aloe

(In writing to advertisers, please mention the Journal—it helps.)

use the **VACUTAINER TECHNIC** *for taking blood culture specimens*

• Break seal and loosen but do not remove cap  on **VACUTAINER BOTTLE.** Prepare patient's arm as usual  for a blood culture. Remove longer cotton roll from needle on tube  and insert needle in vein.  When blood reaches opposite end of tubing, pinch tubing at adapter  to stop flow. Remove cap from bottle,  remove shorter cotton roll and plunge adapter through rubber stopper.  Release pinch on rubber tubing  and allow blood to flow into bottle  until 20 cc. are delivered.

• For anaerobic conditions, pinch tubing at adapter end  and remove adapter from stopper. Remove needle from arm,  replace cap on bottle and incubate. 

• For aerobic conditions, pinch tubing at needle end,  remove needle from arm and thrust into long cotton roll.  Release pinch on tubing to allow filtered air to enter bottle. Remove adapter from stopper, replace cap and incubate.

the B-D VACUTAINER® CULTURE BOTTLE

containing Trypticase Soy Broth with CO_2 added

ASSURES:

- Positive positives and valid negatives
- Absence of outside contamination
- *Elimination of multiple media*
- Faster laboratory handling

Prepared and Sterilized by
BALTIMORE BIOLOGICAL LABORATORY, INC.
Baltimore, Maryland

BECTON, DICKINSON AND COMPANY
RUTHERFORD, N. J.

Send coupon for further details

BECTON, DICKINSON AND COMPANY
Dept. 27-M, 15 Ames Ave., Rutherford, N. J.

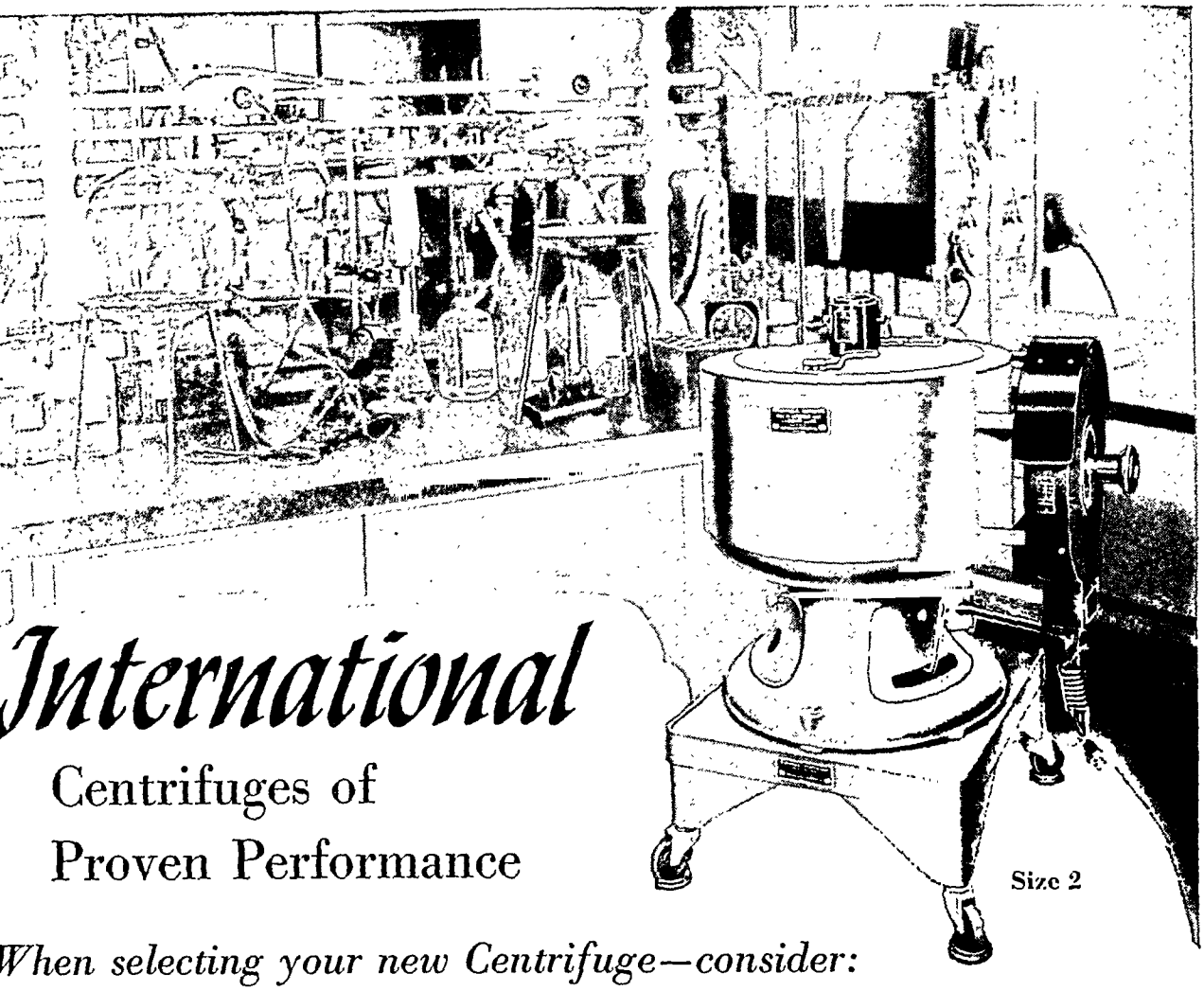
Please send me descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE.

Name_____

Address_____

City_____ Zone____ State_____

(In writing to advertisers, please mention the Journal—it helps.)

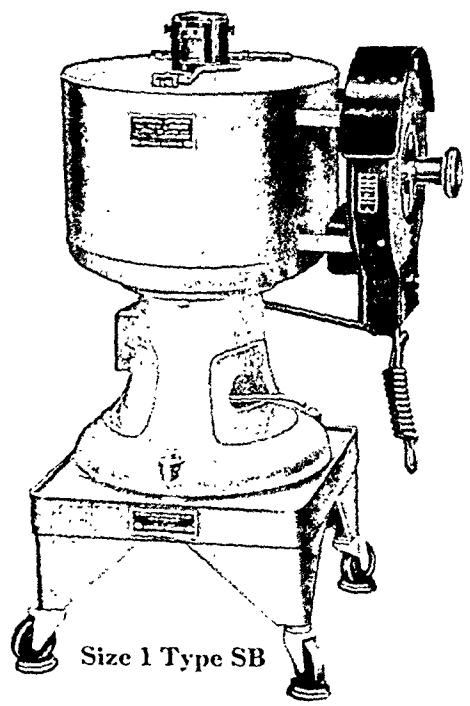


International

Centrifuges of Proven Performance

Size 2

When selecting your new Centrifuge—consider:



Size 1 Type SB

1 **The reliability of the electric motor that drives it.**
The motors of International Size 1 and Size 2 Centrifuges are specially designed to provide the best possible drive for a Laboratory Centrifuge. They are manufactured entirely in our own shops, thus eliminating the possibility of obsolescence through discontinuance by an "outside" manufacturer.

2 **The range of its application.**
Interchangeable accessories designed specifically for International Centrifuges are available in wide variety, making the machines adaptable to practically every laboratory application. Your future requirements as well as your present needs should be considered.

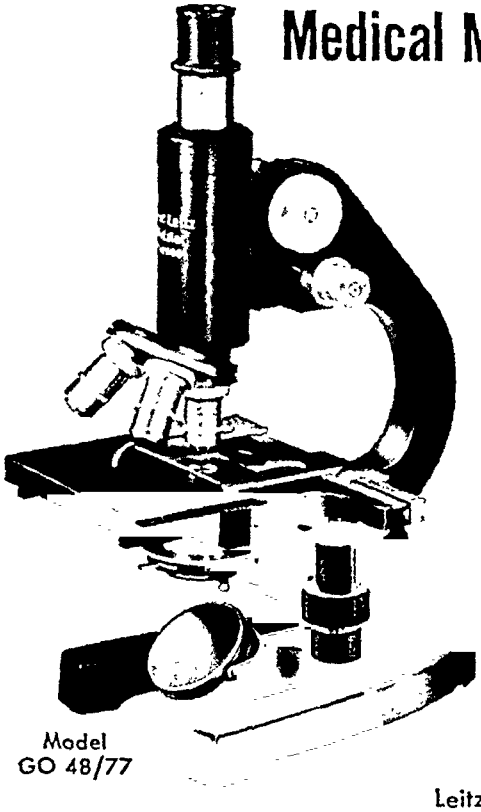
3 **The relation of first cost to total cost.**
Expensive repairs can make a low first cost a high cost in the long run. International Centrifuges are famous for their ability to give years of trouble-free service.

4 **The reputation of the manufacturer.**
International's fifty years of specialization in the manufacture of Laboratory Centrifuges is your guarantee of unequalled performance.

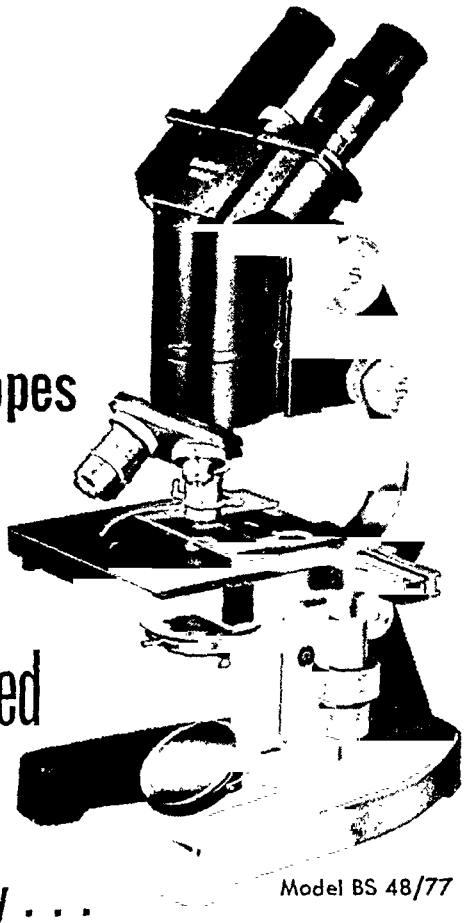
INTERNATIONAL EQUIPMENT COMPANY

1284 SOLDIERS FIELD ROAD, BOSTON 35, MASS.

the NEW Leitz Medical Microscopes



Model GO 48/77



Model BS 48/77

Designed for greater accuracy . . . easier operation

With all the precision and quality traditionally associated with the name Leitz, these improved Medical Microscopes embody the latest advancements optical science can provide. Stands are larger, yet *lighter, with a longer bearing surface for the focusing* motion. Both coarse and fine adjustment are easier working, offer greatest accuracy yet devised. New, built-in mechanical stage with low-set drives on a common axis, traversing area 3" x 2", means easier, smoother operation. New substage condenser with swing-out upper element permits the use of all lenses from lowest to highest magnifications. The oil immersion lens 1/12th, N.A. 1.30, 100x is anti-reflection coated for maximum definition and better color rendition.

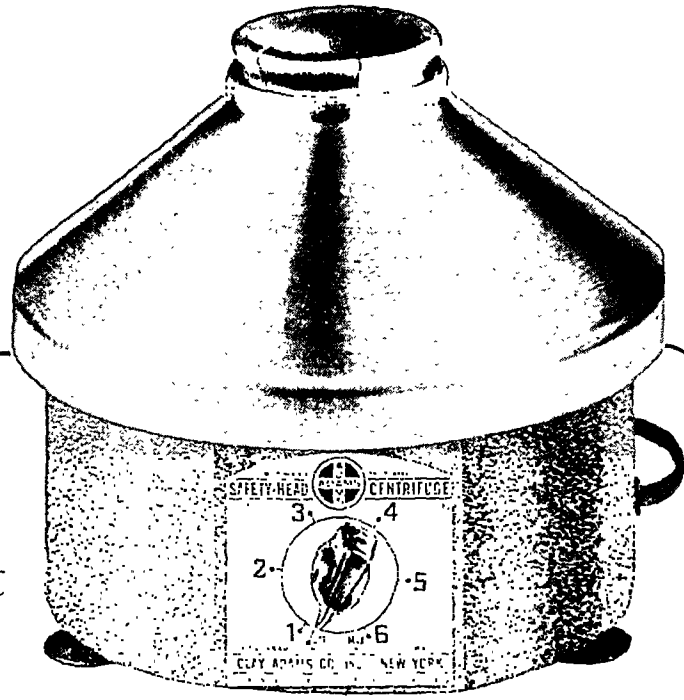
Before you choose, ask your Leitz Dealer for a demonstration of the new Leitz Medical Microscopes.

Write today for Catalog Micro—101P

E. LEITZ, Inc., 304 Hudson St., New York 13, N. Y.

LEITZ MICROSCOPES • BINOCULARS • SCIENTIFIC INSTRUMENTS
LEICA CAMERAS AND ACCESSORIES

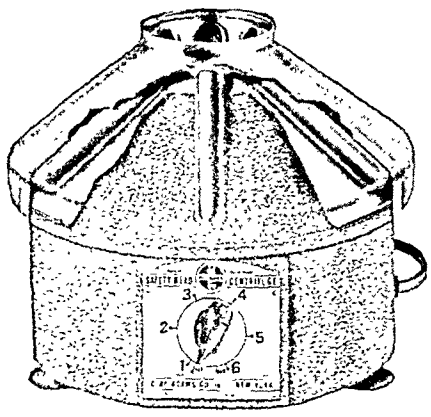
(In writing to advertisers, please mention the Journal—it helps.)



Especially recommended for Wintrobe Hematocrit determinations

ADAMS SAFETY-HEAD CENTRIFUGE

- A compact, lightweight centrifuge which **will fit inside a small refrigerator when centrifuging in the cold is required.**
- Accommodates six 15 ml. tubes . . . **Can be used for micro and semi-micro work** by substituting the proper shields and tubes.
- Maximum speed fully loaded is **4000 RPM on AC, 4500 RPM on DC.** Provides a faster sedimentation rate and more complete sedimentation with finely dispersed particles by use of the angle principle—suspending the tubes at a fixed 52° angle.



CT-1002 with safety cap removed and head cut away to *show position of tubes* and underguard.

Housing is cast aluminum, attractively finished in gray hammertone. Head is made of a special aluminum alloy and precision machined for accurate balance. Rheostat has an "off" position and allows for continuous speed control. Rubber suction feet absorb vibration and keep the centrifuge from creeping, even on glass.

CT-1002 Adams Safety-Head Centrifuge, 6 Place; complete with head, safety cap, underguard, shields and heavy duty glass tubes; for 110 volts, AC-DC. **each $72.00**

220 volt model, $3.00 additional

Write for our Circular 309-CP describing the complete line of Adams Centrifuges

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

ADAMS

MALLINCKRODT

sunlight, month after month



$AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$

Silver Nitrate's traditional sensitivity to light is not inherent. It arises from various impurities. The purest Silver Nitrate, expertly made and carefully handled, withstands prolonged sunlight.

Lot after lot—not sometimes but always—Mallinckrodt Silver Nitrate A.R. withstands sunlight. It is consistently the fine reagent ACS specifications require.

Like all Mallinckrodt Analytical Reagents, Silver Nitrate A.R. assures you of the utmost Uniform Dependable Purity.

ALWAYS SPECIFY MALLINCKRODT REAGENTS IN ORIGINAL PACKAGES

FINE CHEMICALS Mallinckrodt SINCE 1867

82 Years of Service to Chemical Users

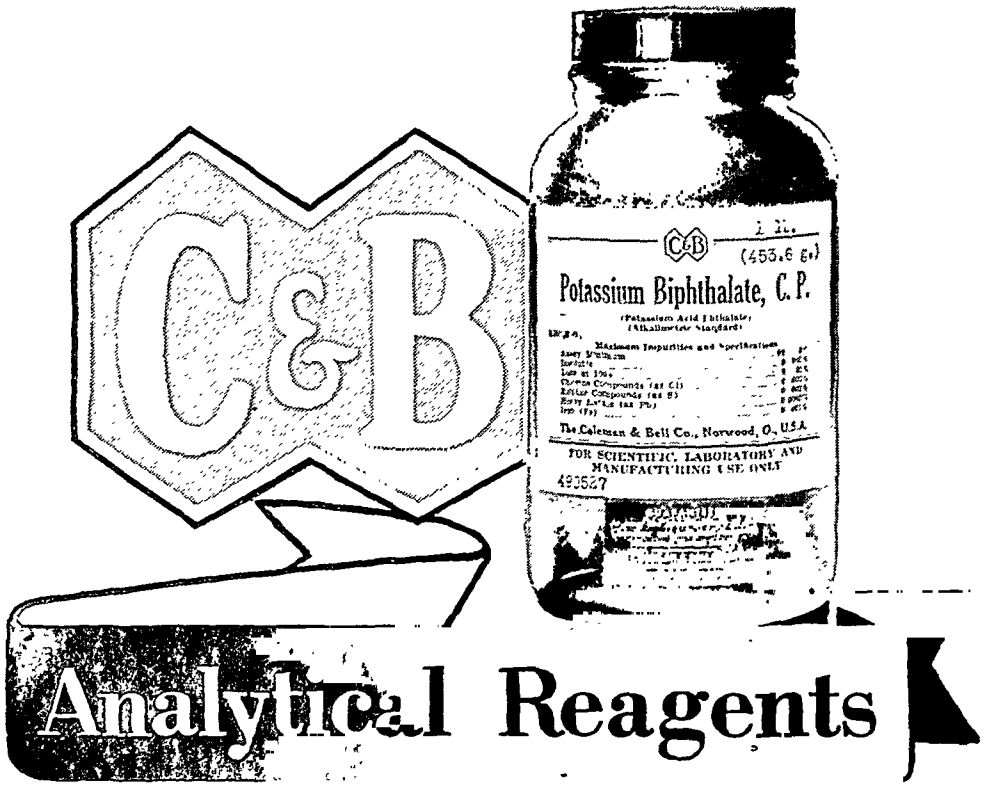
MALLINCKRODT CHEMICAL WORKS

Mallinckrodt Street, St. Louis 7, Mo. • 72 Gold Street, New York 8, N. Y.

Chicago • Cincinnati • Cleveland • Los Angeles • Montreal • Philadelphia • San Francisco

UNIFORM DEPENDABLE PURITY

(In writing to advertisers, please mention the Journal—it helps.)



Analytical Reagents

Potassium Biphthalate, C.P., A.C.S. is one of several hundred different reagents which we manufacture.

C & B Reagents meet definite standards of purity—standards which are adequate to insure products suitable for research and analytical use.

C & B Products are distributed by
Laboratory and Physician Supply Houses
throughout the World

•

Write for a Copy of Our Catalog

•

The COLEMAN & BELL CO., Inc.

Manufacturing Chemists : Norwood, Ohio, U. S. A.

COLEMAN & BELL

(In writing to advertisers, please mention the Journal—it helps.)

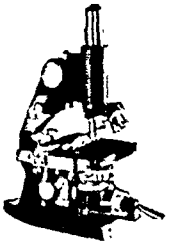
Latest in a Long Line of *Distinguished Spencer Microscopes*



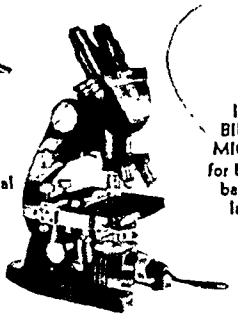
This completely new line of microscopes continues the tradition of quality established in 1847 by Charles A. Spencer, the first American microscope maker. You are cordially invited to see and try them. Ask your distributor or write Dept. Z16.



LABORATORY MICROSCOPES for school and college use.



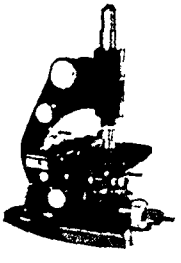
MONOCULAR MICROSCOPES for medical, industrial, educational laboratories.



INCLINED BINOCULAR MICROSCOPES for biological and bacteriological laboratories.



CONVERTIBLE MICROSCOPES, binocular-monocular for advanced work.





MOLD COUNT MICROSCOPES for food processing.

BACTERIA COUNT MICROSCOPES for dairy industries.

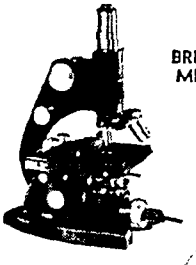
PATHOLOGIST'S MICROSCOPES







WATER AND SEWAGE MICROSCOPES



BREWMASTER'S MICROSCOPES

TEXTILE MICROSCOPES

American Optical COMPANY
Instrument Division
Buffalo 15, New York

SPENCER

(In writing to advertisers, please mention the Journal—it helps.)

SERVING SCIENCE *through supply*

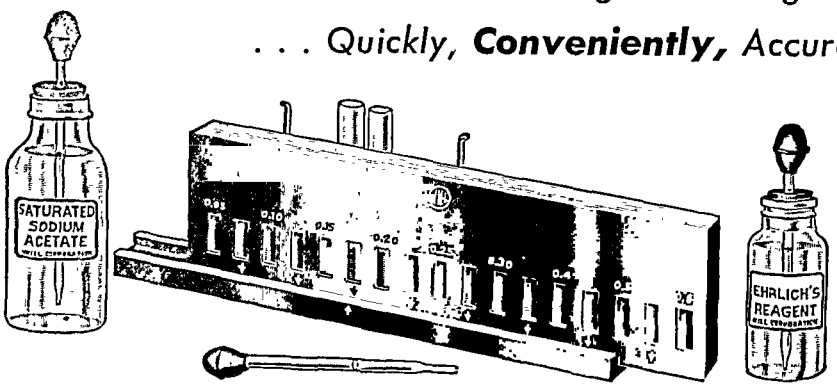


WILL



You'll Like This Comparator Set

Designed for the Specific Job of
Determining Urobilinogen
. . . Quickly, ***Conveniently,*** *Accurately!*



THIS EXCLUSIVE WILL ITEM fills the need for simplified equipment for semi-quantitative determination of Ehrlich urobilinogen in urine and stool . . . useful in differential diagnosis and in following the course of certain diseases. Comparator has base and slide of molded plastic. Color standard slide contains nine sealed standards of pontacyl dye. Color densities are stable and accurately correspond to those developed in the Ehrlich reaction. Bottles have rubber-bulb pipettes for transfer of Ehrlich reagent and sodium acetate solution.

17185—UROBILINOGEN COMPARATOR SET. 9 standards, one 6-oz. bottle and 1.25 ml. calibrated pipette for Ehrlich's reagent, one 12-oz. bottle and 2.5 ml. calibrated pipette for sodium acetate solution. Complete with directions $21.50

17186—EXTRA DROPPING PIPETTES for 17185 Comparator Set. Capacity 1.25 ml. or 2.5 ml. 30c each

ORDER YOURS TODAY: Write, Teletypewrite, Wire or Phone Dept. JCP-12 Our Nearest Office-Warehouse

We Stock Chemicals for the Clinical Laboratory:
"Bioloid" Paraffin.
"Difco" Media.
Stains and Indicators.
Clinical Solutions.

WILL CORPORATION
LABORATORY APPARATUS AND CHEMICALS
ROCHESTER 3, N.Y. • NEW YORK 12, N.Y.
SOUTHERN SCIENTIFIC CO., INC., ATLANTA 3, GA.
BUFFALO APPARATUS CORP., BUFFALO 5, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)

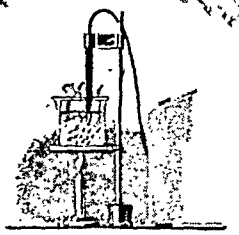


VIRTUALLY UNBREAKABLE!

So strong that the pH-sensitive bulb can withstand as much pressure as the thick stem itself!

Abrasion resistant—unusually rugged for long life and reliable measurement even in highly abrasive slurries!

Chemical durability—excellent in the strongest acids and alkalis . . . even at elevated temperatures!

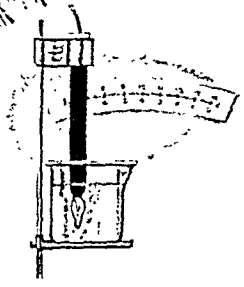


COVER EXTREME TEMPERATURES!

30° to 130° C at high pH—with the new high temperature—high pH glass.

—20° to 100° C—with the new general-purpose glass. Will withstand even repeated freezing.

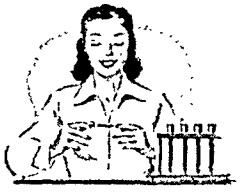
Now- NEW BECKMAN pH Electrodes



UNPRECEDENTED pH RANGE!

0 to 14 pH—with the high-temperature high-pH glass . . . *only small reproducible deviation near extreme limits, even at high temperatures.*

0 to 11 pH—at room temperature and 0 to 10 pH at boiling for the general-purpose glass . . . *without correction for sodium ions!*



IMPROVED CONSTRUCTION!

Simplified, heat-resistant construction eliminates wax filling . . . retains patented Beckman internal shielding . . . increases measurement precision.

Only *Beckman Glass Electrodes* offer all of these features:

▶ **RAPID RESPONSE** . . . accuracy with speed and convenience.

▶ **STABILITY** . . . to match the high precision of BECKMAN pH METERS.

▶ **INTERNAL SHIELDING** . . . gives complete freedom from outside electrostatic interference.

▶ **INTEGRAL LEADS** . . . Continuous insulation into the electrode . . . connections located on protected panel board.

▶ **EXTREME TEMPERATURE RANGE** . . . Several types permit use from below freezing to boiling *and above!*

▶ **ENTIRE pH SCALE** . . . *Very small* sodium ion errors . . . e.g., with high-temperature high-pH glass, only 0.2 pH deviation at 13.7 pH in 1 N sodium hydroxide.

▶ **FACTORY SEALED** . . . rigidly tested . . . no maintenance.

▶ **UNIQUE CHEMICAL DURABILITY** . . . in alkali . . . in acid . . . in dilute solutions.

And . . . *REALLY TOUGH* . . . can even be used as a stirring rod!

Constant Research over the years has maintained Beckman Leadership in the instrumentation field . . .

—Beckman pH Meters and Glass Electrodes
—Beckman Ultraviolet, Visible and Infrared Spectrophotometers
—Beckman Radioactivity Meters and Special Instruments . . . Unsurpassed for Reliability!

These new electrode glasses are now being used in several Beckman electrode styles. As rapidly as possible, they will be available in the full line of Beckman Glass Electrodes.

BECKMAN INSTRUMENTS control modern industries

ECKMAN INSTRUMENTS • National Technical Laboratories, South Pasadena 44, California

FIVE

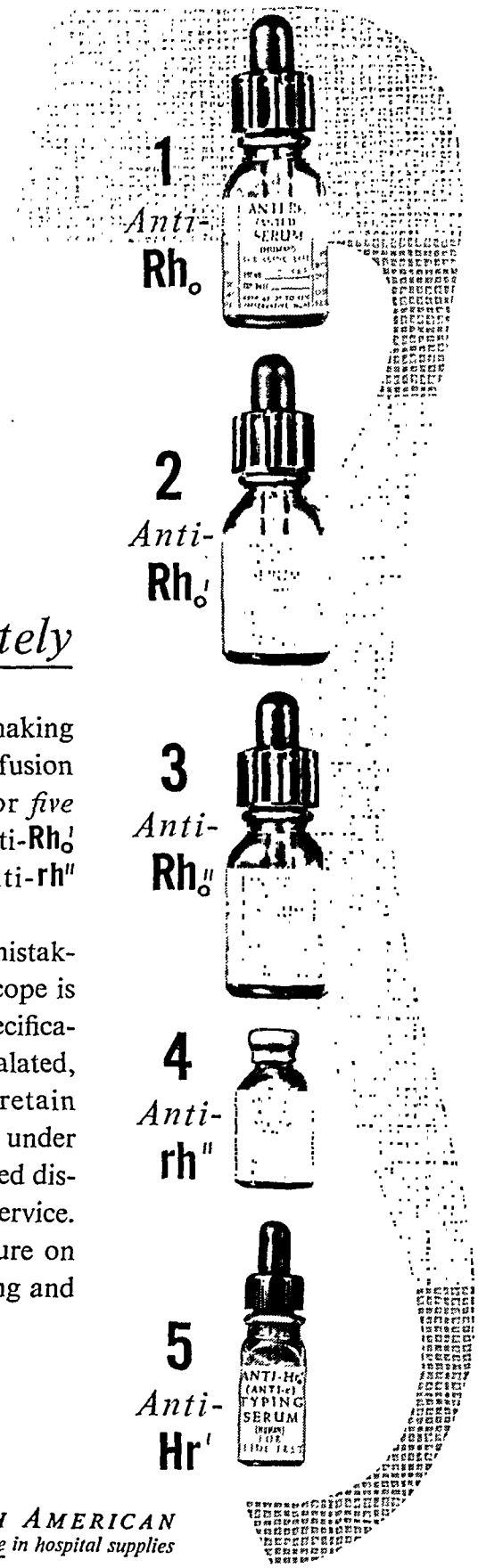
Anti-(Rh) Serums

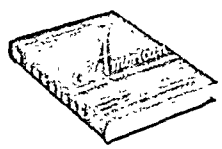
to test every blood—completely

More and more pathologists are making *complete* **Rh** determinations for blood infusion cases. They're turning to American for *five* potent serums—Anti-Rh_o (Anti-D), Anti-Rh_o^I (Anti-CD), Anti-Rh_o^{II} (Anti-DE), Anti-rh^{II} (Anti-E) and Anti-Hr^I (Anti-c).

The slide method gives accurate, unmistakable readings in two minutes. No microscope is needed. Serums, prepared to N. I. H. specifications, may be used with fresh, clotted, oxalated, heparinized or citrated blood. They retain original potency over a long period even under adverse conditions. Six strategically located distributing centers assure you of prompt service.

WRITE today for descriptive literature on AMERICAN'S complete line of blood-typing and blood-grouping serums.





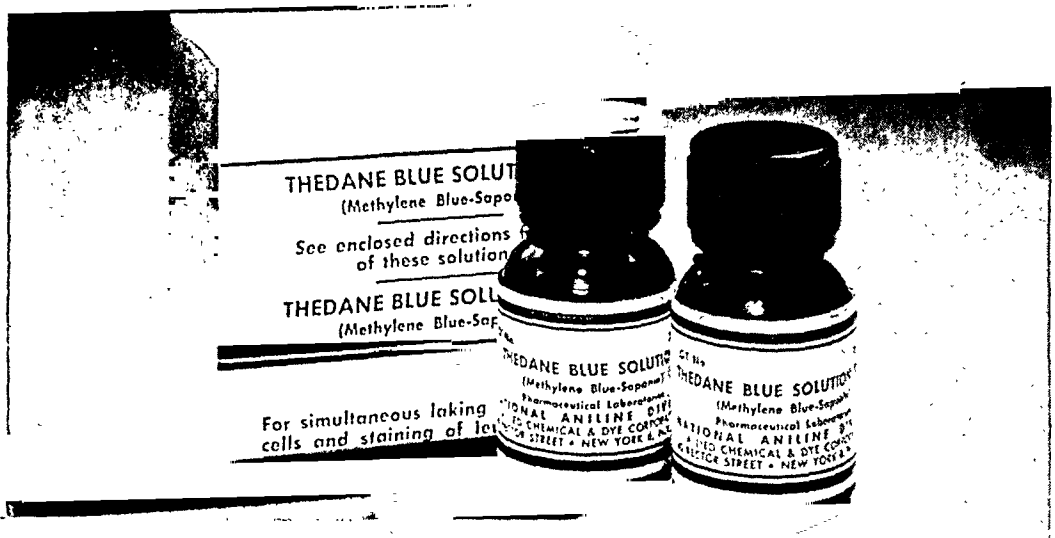
PLAN WITH AMERICAN
... the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

First American Production of

Another New **National** *Stain*



THEDANE BLUE

SOLUTIONS T-3 AND T-5

for the Rapid and Precise Identification of Blood Parasites

Thedane Blues are very stable, tropic-proof solutions for demonstrating trypanosomes, malarial crescents, piroplasms, blood spirochetes, treponemes in syphilitic chancre exudates, leptospirae, leucocytes and reticulocytes. They assure rapid, reliable diagnosis by simultaneous hemolysis and monochromatic staining of the parasites. They have these advantages:

Save time and simplify procedure thru ultra-rapid staining of fresh blood preparations with undiluted stock solutions. 50 to 100 cu. m.m. (or more) of fresh blood can be prepared in less than 10 seconds.

Instantaneous hemolysis completely clears the image field. Only parasites, leucocytes and reticulocytes are stained.

Only medium or high dry systems are used. Large image fields assure rapid, reliable diagnoses even at lowest parasite densities.

In dark-field preparations, spirochetes and leptospirae show brilliantly red, indicating suspicious spots at medium power. High power verifies diagnosis.

Ideal staining for diagnosis of microfilariae in extremely thick wet blood drops at low power.

Indispensable in bird-malaria research because they make possible examination of thick drops in nucleated erythrocytes.

Very instructive for parasitological and hematological class room work.

Leaflet giving detailed data on applicability and method of use sent on request.

Pharmaceutical Laboratories

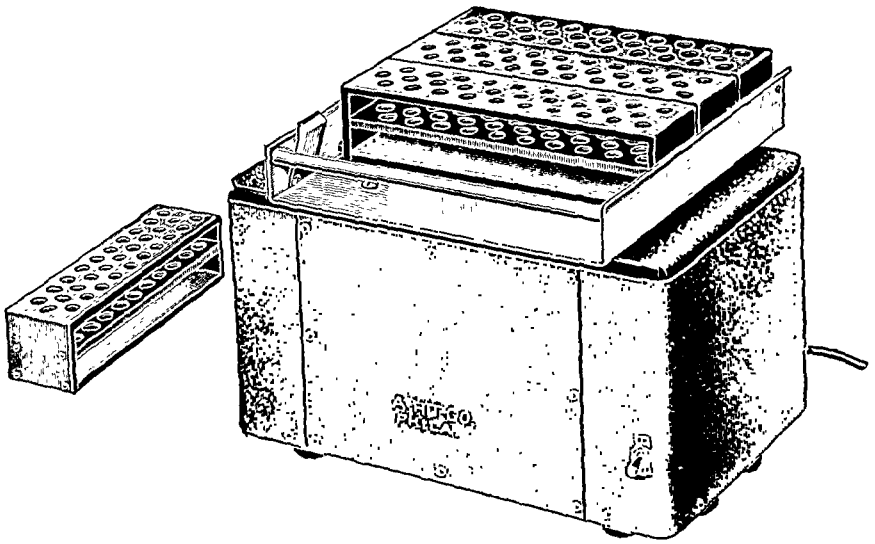
NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A.

IMPROVED MODEL

KAHN SHAKING APPARATUS

With 4-rack carrier of anodized aluminum, enclosed power unit, and Neoprene covered racks



8915.

KAHN SHAKING APPARATUS, Improved Model. With power unit adjusted to produce 275 to 285 oscillations per minute with horizontal stroke of 1½ inches, as specified by the author. See R. L. Kahn, *"The Kahn Test, A Practical Guide", (1928), p. 66.*

Power unit consists of a ¼ h.p. split phase induction motor connected to a drive wheel by means of a ⅜-inch V-belt, in sheet metal housing, 16½ inches long x 11½ inches wide x 9¾ inches high, with crystal black finish and eight rubber suction feet to dampen vibrations. Power consumption, 500 watts.

Carrier accommodates four Kahn racks and is constructed of anodized aluminum, with spring clips which permit insertion of racks firmly in position without use of adjustable bars or clamps, and removal of one or more racks without disturbing adjacent racks.

8915. **Shaking Apparatus, Kahn, Improved Model,** 4-rack, as above described, complete with four Neoprene covered Kahn racks, on-off switch, 6 ft. connecting cord and plug, and directions for use. For 115 volts, 60 cycles, a.c..... **106.56**

8915-C. Ditto, but without Kahn racks..... **92.00**

NOTE—A six-place carrier for two rows of three Kahn racks each is available at $30.00; also interchangeable carrier for fifteen 250 ml Erlenmeyer flasks at $55.00; for twelve 500 ml flasks at $55.00, and a general purpose box-type carrier at $20.00.

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U.S.A.

CABLE ADDRESS, "BALANCE", PHILADELPHIA

(In writing to advertisers, please mention the Journal—it helps.)

THE PATHOLOGY OF CHEMICAL CARCINOGENESIS*

HAROLD L. STEWART, M.D.

From the Pathology Section, National Cancer Institute, National Institutes of Health, United States Public Health Service, Bethesda 14, Maryland

The first instance of cancer due to a specific agent observed in man was described in 1775 by Sir Percival Pott. He traced the cause of skin cancer in chimney sweeps to the associated contamination of the skin by soot. After this first astute observation, the relationship between cancer and carcinogenic agents lay unproven until less than four decades ago.

Many experimentalists had tried to induce cancer in animals by the application of chemical agents, but success was not attained until 1915 when Yamagiwa and Ichikawa applied solutions of coal tar to the skin of the rabbit's ear. During the years 1921–1926 Block in Zurich demonstrated that the active substance of coal tar is concentrated in the fractions with higher boiling points, probably belonging to the class of cyclic hydrocarbons. Kennaway in England in 1924–25 was able to show by a series of experiments that carcinogenic agents in tar are complex hydrocarbons of the aromatic type. The fluorescent properties of these hydrocarbons proved a useful tool in selecting from a variety of chemically pure compounds one substance that gave a fluorescence spectrum which was similar to that of tar. This was found by Hieger to be 1:2 benzanthracene.

Kennaway and Hieger then tested a variety of other benzanthracene hydrocarbons. Positive results were obtained with 1:2:5:6 dibenzanthracene. To date about 300 chemical carcinogens have been synthesized, of which several possess powerful carcinogenic properties.

One such is 20:methylcholanthrene, used experimentally to produce skin cancer after painting, or sarcomas after subcutaneous implantation. Gastric cancer in mice has been induced following the injection of this substance into the wall of the stomach. Following oral administration to mice, carcinoma of the forestomach and of the small intestine develop. Another synthetic hydrocarbon is 5:9:10-trimethyl, 1:2-benzanthracene which, besides evoking skin cancer or subcutaneous sarcoma, causes the production of pigmented cutaneous lesions. These melanin aggregates are not true melanomas, for it has been demonstrated that by bleaching the melanin-containing cells they can be identified as macrophages which have ingested melanin. This excessive production of melanin is probably due to the stimulation of melanoblasts in the epidermis by the carcinogenic agent. Recently, Berenblum has succeeded in inducing a malignant metastasizing melanoma in a guinea pig painted with 9:10 dimethyl, 1:2 benzanthracene.

In addition to polycyclic hydrocarbons, there are other pure chemical substances which induce cancer in animals. Among these may be mentioned the

* Presented at the Twenty-Eighth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1949.

Received for publication, September 10, 1949.

AZO dyes, some of which selectively cause tumors of the liver when fed to rats and mice. Another example is beta-naphthylamine, one of the intermediates thought to be responsible for cancer of the urinary bladder in dye workers, and now known from the experiments of Hueper to be capable of inducing similar tumors in dogs.

We may add to the list of industrial hazards the recent discovery of a higher-than-normal incidence of lung cancer in chromium workers, and the production of bone tumors following intravenous injections of beryllium in animals. These observations indicate that in industrial establishments some organic metals as well as the hydrocarbons are potentially dangerous.

Beginning with the experiments of Lacassagne in 1932, a few of the hormones have been shown to be carcinogenic. Interstitial cell tumors of the testis can be produced in mice by the subcutaneous implantation of the synthetic estrogen, diethyl-stilboestrol. Analysis of the hormonal content of normal and neoplastic interstitial cells is now being carried out at the National Cancer Institute. The relationship between the milk factor, hormones and breast cancer in mice is also being investigated.

2-acetyl-amino-fluorene, a compound first investigated for insecticidal activity, has been shown to be carcinogenic for a wide variety of animal tissues. It readily provokes liver-cell tumors in rats and occasional tumors of the salivary glands, the thyroid gland, the bladder, and the mammary glands in this species. Because of this widespread action, it has proved to be very useful in experimental cancer research.

In summary, a few of the uses being made of carcinogenic agents in work now going on at the National Cancer Institute have been described. By means of these substances we hope to be able to identify the factor or factors which may be essential in the production of neoplasms, and also to determine what the earliest stages of cancer look like, so that neoplastic disease may be detectable in a curable stage. Finally, we hope some day to ascertain the underlying characteristics which are peculiar to cancer cells. This calls for the collaboration of all the medical sciences, as well as the disciplines of physics, biochemistry, radiology, nutrition and others. Only by such an all-out attack will the nature and origin of cancer be determined. It can be seen that the problem of tumor induction by carcinogenic agents touches the very core of the problems of growth and life.

SOLITARY "TUMORS" OF ATYPICAL AMYLOID (PARAMYLOID)*

WILLIAM H. BAUER, M.D., AND J. F. KUZMA, M.D.

From the Department of Pathology Saint Louis University, Schools of Medicine and Dentistry, and the Division of Laboratories, Milwaukee County Hospital, and Department of Pathology, Marquette University, School of Medicine

Amyloidosis is usually considered to be a degenerative disease characterized by the presence in mesenchymal tissue of a complex albuminous substance composed of protein fractions and a sulfate-bearing polysaccharide. It frequently involves blood vessels and the supporting tissue of the viscera; however, it may also be found in completely mesodermal tissues, such as muscle, bone, and fat.

REVIEW OF LITERATURE

Based on Askanazy's[2] and Lubarsch's[22] classification of amyloid diseases, Koletzky and Stecher[19] proposed a subdivision into four groups: (1) secondary amyloidosis following chronic inflammatory and destructive diseases, (2) primary amyloidosis without known etiology, (3) amyloidosis associated with multiple myeloma, and (4) tumor-forming amyloidosis.

Primary or, preferably, atypical amyloid disease (called by Picchini and Fabris,[27] Strauss,[33] and others paramyloid disease) occurs rarely. In a recent study Iverson and Morrison[17] increased the number of the reported cases to forty-four. King,[18] who suggested a subdivision into typical and atypical amyloidosis, found approximately 50 cases of the atypical variety in the literature. Recently, Dahlin[9] collected 57 cases including his own. The atypical amyloid disease is of unknown etiology. It is found chiefly in mesenchymal tissue, such as striated and smooth muscle (gastrointestinal) but generally not in the liver, spleen, kidney, or adrenal. However, there may be systemic involvement or, in rare cases, only a local disturbance affecting one or two organs. In such cases, the atypical amyloid shows a faint or completely negative reaction to special stains. This fact may be accounted for by the variability of the chemical composition of amyloid as shown by von Bonsdorff,[5] Hass and associates,[14] and recently by Randerath.[28] Occasionally both atypical and typical amyloid may be found together.

Gordon[13] noted the formation of atypical amyloid in rheumatic fever. He described it in the heart, valvular vegetations, and in the small intestine, while typical amyloid was present in the kidney, liver, and spleen. This observation is in accord with other reports of atypical amyloid changes in rheumatoid arthritis (Yeoman and Wilson,[39] Trasoff and associates[35]). It has also been observed that cases of multiple myeloma may display a combination of atypical and typical amyloid (Atkinson[3]), although the atypical variety is more common. A substance resembling amyloid, found by Teilum[34] in cases of Boeck's sarcoid, Letterer-Siwe's disease, and in some instances of allergy to sulfonamides, seems to

* Received for publication, July 25, 1949.

belong in this category. Teilum[34] did not hesitate to call it paramyloid. King[18] described atypical amyloid in the hearts of old people.

In some cases of nodular multiple myeloma and diffuse myeloma (Freund[11]), atypical amyloid accumulated in the bone marrow and caused a tumor-like expansion of bone, perforation of the cortex, and involvement of the local periosteum, fat and muscle tissue. This bone involvement was found to be associated with systemic amyloidosis or, as in Freund's[11] case, with lesions restricted to a few bones. However with both types of distribution, the presence of myeloma cells indicated the underlying disease. Different from the cases of myeloma with amyloidosis is the rare instance of bone involvement in primary systemic amyloidosis as described by von Bonsdorff,[5] and Koletzky and Stecher.[19] In the case of Koletzky and Stecher the valves of the heart, the joints and the bones were affected, and the patient suffered a pathologic fracture of the left femur.

Although there are a few reports in the literature dealing with local deposition of atypical amyloid in malignant tumors (as in osteogenic sarcoma and renal carcinoma) and also in hemangioma, solitary "tumors" composed of atypical amyloid involving soft tissue or one or two bones without discoverable cause are extremely rare. Some authors doubt that such growths occur and claim that a thorough examination would reveal an underlying myeloma (Freund,[11] Magnus-Levy,[23] Rosenblum and Kirshbaum[29]). Nevertheless reports in the literature[7, 10, 15, 16, 24, 30, 36] and our own studies establish the existence of local tumor-accumulations of an amyloid-like material in bones and soft tissue without obvious cause. By critical study Bürgi[7] arrived at the conclusion that the cases reported by Hedren[15] (ninth and tenth ribs), Mandl[24] (thoracic vertebra), Hildebrand[16] (sternum), Schmidt[30] (maxilla), and Vogeler[36] (skull) should be regarded as local amyloid "tumors" of unknown origin. Bürgi's[7] own case (parietal bone) and Duenner's[10] case (sternum) complete the list.

REPORT OF CASES

Case 1

Clinical data. A 57 year old white bartender, moderately obese, was admitted to hospital on February 10, 1944 and expired on February 20, 1946. He had had increasing difficulty in walking over a period of six to eight months. One week before admission, walking was particularly difficult and he fell. Since that time he had not been able to stand or walk. In the weeks preceding admission to the hospital he also developed a prickling sensation in the soles of the feet and spontaneous jerking contractions of the lower extremities. The patient was free of pain until after the fall when he noticed pains in the shoulders and in the lower anterior portions of the chest, which were considerably exaggerated by coughing. Disturbances of micturition also developed following the fall. The past history and the family history contributed no pertinent information.

The only positive findings on physical examination were those of moderate enlargement of the prostate and flaccid atrophic muscles of the legs. These muscles were not tender. The thigh groups of muscles were quite tight. Spontaneous contractions of various muscles with uncoordinated movements were noted. Babinski's and Oppenheim's signs were present bilaterally. Touch and pain sensations varied considerably but all examiners felt that there was some change from normal. There was slight kyphosis with tenderness over the ninth dorsal area. Examinations of the heart, lungs and abdominal organs were without noteworthy findings.

Roentgenographic examinations of the chest, gastrointestinal tract and skull were negative. Examination of the spine, however, revealed a marked compression of the ninth dorsal vertebra associated with changes in the longitudinal muscle groups. This was interpreted as a possible abscess in the muscles.

Spinal fluid obtained by lumbar puncture was under normal pressure and revealed slight xanthochromia. The flow stopped rather suddenly and there were no changes in the pressure noted upon coughing or upon compression of the jugular vein. Examination of the fluid revealed no increase in cells, 4+ globulin, and total protein of 800 mg. The serologic test for syphilis and the colloidal gold reaction were negative. Cerebrospinal fluid obtained by cisternal puncture was clear, colorless and under normal pressure. Total protein was 25 mg., sugar 62.4 mg., chlorides 720 mg., and globulin 1+.

On April 8, 1944 a laminectomy was performed. Soft, brown, granular, friable tissue was found protruding from the vertebral body compressing the spinal cord. The tissue was removed and cord pulsations returned, but no clinical improvement followed the operation. During the following year, the patient developed cervical adenitis which subsided rather quickly and there was a temporary paralysis of the right abducens nerve. He also survived an episode of pneumonia for which he received sulfadiazine therapy. On February 19, 1946 the patient complained of headache and stiffness of the neck. Temperature was 103 F. The next day the patient had convulsions and expired.

Laboratory findings were as follows: sedimentation rate, Westergren method, 4 mm. in one hour and 10 mm. in two hours; red blood cell count generally low, averaging about 3,700,000; white blood cell count from 7000 to 25,000, the latter at the time of the pneumonia; differential blood count not unusual; on December 20, 1944, 2 per cent band forms, 49 per cent segmented forms, 4 per cent eosinophils, 2 per cent basophils, 2 per cent monocytes, and 41 per cent lymphocytes; no abnormal haziness of the blood films or autoagglutination of the red blood cells; nonprotein nitrogen on December 14, 1945, 35.3 mg. per 100 ml. blood; acid phosphatase 8.4, 10.4, and 8.4, and alkaline phosphatase 17.0, 10.8, and 15.4, King-Armstrong units; multiple urinalyses consistently negative except for presence of sulfonamide crystals on several occasions; Bence-Jones protein test negative twice; no albuminuria at any time; serologic tests for syphilis consistently negative; multiple samples of sputum and gastric aspirations for tubercle bacilli negative. No total protein or blood calcium determinations were made, and no sternal marrow was aspirated, but postmortem marrow was studied.

Autopsy examination of the base of the skull showed marked softening and friability of the body of the sphenoid bone and adjacent portions of the occipital bone. These areas were reddish gray and were composed of many smooth, minute, sand-like particles. The tissue was dry and crumbled. No other skull changes were found. There was no suppuration in the middle ears or paranasal sinuses. The ninth dorsal vertebra was collapsed so that the intervertebral discs were almost approximated. The substance of the vertebra was reddish brown, very soft, friable and difficult to handle. The adjacent ligamentous tissue was firm and partially calcified. The left ninth rib just lateral to its angle presented a fusiform swelling which was somewhat lobulated and measured 5 cm. in length. Both internal and external surfaces of the rib were very thin and partially destroyed. The bulk of the swelling was composed of soft, granular, grayish red tissue of a sandy friable nature. The remainder of the thoracic cage, spine, and pelvis showed no lesions. The extremities were not examined, but roentgen investigation showed no lesions. Death was caused by the development of a purulent meningitis for which the portal of entry may have been through the sphenoid sinus involved by the paramyloid. Additional findings were those of coronary and

renal arteriosclerosis, moderate prostatic hypertrophy and diverticulum of the urinary bladder. The liver weighed 1410 grams, the spleen 90 grams, and the combined weight of the kidneys was 250 grams. There was no gross evidence of amyloid disease in the viscera and histologically all of the viscera failed to show any type of amyloid.

Case 2

Clinical data. A well-developed, well-nourished, 53 year old white woman presented herself with the complaint of a nodule in the anteromedial aspect of the distal third of the left thigh. This nodule was first noticed fourteen years previously as a small, pea-sized, movable, firm, painless lump just beneath the skin. She had rather vague recollection of a severe bump to this area four years before the tumor was noticed. At about the same time the patient became aware of varicose veins of the same extremity. The tumor gradually enlarged in the course of these years to the size of a golf ball, at which time it became less movable and apparently was attached to the skin. One year ago the patient had received treatment for varicose veins which then disappeared. The nodule, however, remained and the patient sought medical advice concerning its removal. A physician had thought it to be a lipoma. Other past history included draining cervical adenitis of several weeks' duration which responded well to nine roentgen treatments. One year previously she had had her gallbladder removed and developed postoperative pulmonary embolism. There was no other pertinent physical finding at that time and at present the patient is in good health.

During previous hospital admissions and in visits to her private physician, laboratory examinations of blood and urine were normal. Four months after removal of the tumor, laboratory data were as follows: blood hemoglobin 15.5 grams; color index 0.93; erythrocytes 5,400,000; and leukocytes 13,450 with segmented forms 62 per cent, stab forms 5 per cent, lymphocytes 26 per cent, monocytes 4 per cent, and eosinophils 3 per cent; no excessive rouleaux formation or hazy film; erythrocytes normal; sedimentation rate (Westergren) 23 mm. in one hour; nonprotein nitrogen 28.3 mg. per 100 ml. blood; calcium 11.9 mg.; phosphorus 3.3 mg.; total protein 7.30 Gm.; albumin 4.05 Gm.; globulin 3.25 Gm. (sodium sulphite method); Takata-Ara test, cephalin-cholesterol flocculation, blood Wassermann test, and urinalysis all negative; sternal marrow, normal cytology with an occasional lipoid-filled histiocyte.

Roentgen studies of the skull, spine, pelvis, and ribs were entirely negative.

Microscopic examination of the "tumors". Celloidin sections were treated with hematoxylin and eosin and van Gieson stains, paraffin sections with Bennhold's Congo Red stain, and Mallory's crystal violet method as modified by Lillie.

In Case 1 innumerable spherical and bizarre bodies were densely scattered through the highly osteoporotic sphenoid and the left ninth rib. In the rib and sphenoid masses of variable size were dispersed within a diffusely infiltrating substance which formed droplets or rivulets (Fig. 1). It was evident that the large spherical bodies were shaped either by concentric deposition, proved by the

FIG. 1. Case 1. Pattern of interior of sphenoid bone showing complete absence of bone trabeculae. The area is occupied by small and large, confluent, grape-like masses of paramyloid about which are bizarre giant cells. The interstitial tissue consists of proliferated reticulum cells and scattered lymphocytes. Strands of this tissue are entrapped in paramyloid. H and E. × 200.

FIG. 2. Case 1. Portion of sphenoid bone showing concentric well-calcified body of atypical "amyloid" (paramyloid) in bone marrow space which is filled with fused droplets of paramyloid that has not yet become calcified. H and E. × 300.

FIG. 3. Case 1. Portion of sphenoid bone showing layered "paramyloid" mass formed by fusion of small bodies with partial calcification. Note the flattened inner surface of the cortical bone. H and E. × 450.



FIGS. 1–3

arrangement of layers, or by fusion of droplets (Figs. 2 and 3). In hematoxylin eosin-stained sections most of the central parts of the layered deposits and many of the droplets displayed a cloudy blue color while the peripheral zones of the bodies appeared pink. This finding indicated that the central parts were densely calcified and surrounded by a less calcified or noncalcified peripheral zone. In sections stained by the van Gieson method the bodies appeared yellow. Bennhold's Congo Red stained the outer layers lightly red and the core slightly purple. Lillie's modification of Mallory's crystal violet made the outer layers of the bodies appear purple and the central parts bluish. First, we embedded the bone specimens in celloidin, but the results with metachromatic stains were not convincing; later, in using paraffin sections of fresh material we obtained tinctorial reactions of "atypical amyloid" or paramyloid. Henceforth, we shall use these terms.

The scant tissue between the paramyloid and the few remining bone trabeculae consisted of dilated capillaries and free red blood cells between surviving bone marrow cells, among which immature myeloid and reticulum cells were predominant. Macrophages and plasma cells were noted only occasionally. There was an almost complete absence of fat cells, yet an abundance of collagen was seen in van Gieson-stained sections. The larger arteries in the marrow spaces were patent and had unchanged walls, while the smaller arteries and capillaries were affected. The paramyloid bodies either lay free in the scanty remnants of the bone marrow or were closely applied to the lacunar indentations of small fragments of spongy bone-trabeculae and thinned, broken cortex. The most interesting areas were those in which irregularly shaped paramyloid bodies were directly covered by broad layers of a substance which stained bright pink with eosin, but remained colorless in sections treated with Congo Red or Mallory's crystal violet. This homogenous, structureless material, which contained a few entrapped reticulum cells, connected many bodies of atypical amyloid with each other and also with the fragments of old or newly-formed bone trabeculae (Fig. 4). Only here and there a single layer of flat spindle cells appeared interposed between bone and paramyloid body.

With few exceptions, the large and small bodies of atypical amyloid were surrounded by large multinucleated giant cells (Fig. 5). The cortex of the sphenoid and the rib was thinned and perforated in many areas. Though there was a moderate reactive periosteal deposition of bone, paramyloid masses extended

FIG. 4. Case 1. Section of sphenoid bone showing calcified layered paramyloid bodies connected with each other by eosin-staining, hyalin-like substance which contains a few strands of cellular stroma. Calcified paramyloid and hyalin-like substance are in direct connection with a dense irregularly-calcified bone spicule. Note the giant cell embracing the paramyloid body. H and E. × 200.

FIG. 5. Case 1. Section from sphenoid bone showing small eroded island of bone surrounded by eosin-staining hyalin-like, calcified paramyloid. Two adjacent giant cells have hyperchromatic nuclei and disintegrating cytoplasm. H and E. × 300.

FIG. 6. Case 2. Section from mass in left thigh showing trabeculated aggregations of paramyloid with entrapped cells, lymphocytic infiltrate in left lower corner, and a few giant cells in upper central portion. H and E. × 200.

FIG. 7. Case 1. Section of rib showing calcified paramyloid body with fracture. The fracture-space is filled with partly hyalinized connective tissue. The amyloid is, in places, connected with bone by eosin-staining, hyalin-like substance. Innumerable small paramyloid deposits and many giant cells lie in the surrounding tissue. H and E. × 250.









FIGS. 4–7

through openings in the cortex into the periosteum and the epiperiosteal tissue. No paramyloid was found in the periosteal arteries.

The involvement of the ninth dorsal vertebra differed in many features from those described above. There was no remarkable osteoporosis and the ratio between fat cells and other cells was unaltered. However, most of the hemopoietic cells showed postmortem degenerative changes characterized by disappearance of their nuclei and a granular disintegration of the cytoplasm. Among the well preserved bone trabeculae lay numerous, free or clustered, round or polygonal bodies of various sizes. Some of these appeared free in the bone marrow, some were in deep lacunae and were directly connected with the bone surface and some were embedded in the bone trabeculae. In hematoxylin and eosin-stained sections most of this concentrically arranged material appeared deep blue, thereby indicating a calcification more intensive than that in the bone. In contrast to the enormous number of giant cells in the sphenoid and rib only a few of the paramyloid bodies of the ninth dorsal vertebra were surrounded by giant cells. The densely calcified disc between the ninth and tenth vertebrae was thinned in its anterior part and its cartilaginous plates were broken in two areas. In one of these the wide gap extending through the whole thickness of the disc was filled with vascular connective tissue of variable density which contained macrophages, hyalin, cartilage and small concentrically arranged paramyloid bodies.

In Case 2, the tumor was composed of an extremely dense accumulation of round or bizarre layered bodies. With hematoxylin and eosin they stained red in the periphery and bluish in the central layers, yellow with van Gieson, and did not react with Congo Red. However, the Mallory-Lillie technic produced a definite purple stain of the peripheral zones and occasionally of the whole substance. Interspersed among these deposits there was a moderate number of connective tissue cells and vessels, the walls of which consisted of the same cloudy material which we considered to be paramyloid (Fig. 6). The many foci of lymphocytes were striking. The paramyloid extended into the fat tissue. Many of the paramyloid bodies showed intensive calcification brought about by fusion of fine calcium granules in concentric arrangement. The number of giant cells around the paramyloid was moderate compared with the great number of these cells in the first case.

DISCUSSION

Two cases of solitary, tumor-like accumulations of a material which resembled amyloid morphologically, yet displayed unusual distribution and inconstant tinctorial reactions, offered the opportunity to answer the following questions:

1. Is the deposited material a kind of "atypical" amyloid (paramyloid), *i.e.*, atypical in staining reaction and in distribution?
2. What changes does the material undergo?
3. How does the substance affect bone, cartilage, and fat?
4. Is it secondary to a neoplasm or a systemic disease?
5. Does the microscopic examination present a clue to the mode of deposition of the atypical amyloid (paramyloid)?

1. The paramount question is: Was the peculiar substance which accumulated in tumor-like pattern a kind of amyloid? All authors who described atypical amyloid or paramyloid stressed the variability and inconstancy of its staining reaction and many found that in some cases various areas in the same section stained differently. We noted that some of the coarse clumps in the sphenoid and rib of the first case stained with a positive metachromatic reaction while the deposits in the vertebra did not. Gerber[12] stated that in his case the amyloid in the walls of vessels stained deeply with Congo Red while the amyloid masses did not stain. Iverson and Morrison[17] observed a different color in otherwise morphologically identical atypical amyloid. King[18] underscored the inconstancy of the tinctorial reactions of atypical amyloid. Dahlin[9] found that Congo Red stain was not satisfactory and the reddish violet color varied markedly in different areas. In accord with these and other authors we found that there were numerous gradations in the metchromasia of these bodies. This variability of the staining reaction may be due to the different chemical composition of the substance in its developmental phases. Some authors consider the existence of a "preamyloid". We are convinced, and it seems self-explanatory, that the tinctorial reaction of the deposits in our cases was greatly influenced by the unusual calcification of the paramyloid bodies. The irregularity of this granular calcification resulted in different tones of color in each layered paramyloid clump.

The paramyloid appeared as a cloudy, homogenous, concentrically-arranged, hyalin-like substance which contained few entrapped cells. Loeschke,[21] who studied the nature of hyalin and amyloid in rats which were sensitized by injection of 5 per cent nutrose (sodium caseinate), came to the conclusion that all material called hyalin is the product of antigen-antibody reaction, and that amyloid is just a special form of the serologic precipitation of hyalin. Loeschke claimed that amyloid is usually mixed with hyalin substances and, therefore, gives variable staining reactions. In line with this, Teilum[34] referred to the hyalinosis in Boeck's sarcoid and other conditions associated with hyperglobulinemia as paramyloidosis (atypical amyloid). Hyalin is a generic term, whereas amyloid, probably closely related to it, denotes a more or less characteristic staining reaction.

We observed in hematoxylin-and-eosin-treated sections bands of a bright pink homogenous material extending between paramyloid bodies and newly-formed bone trabeculae. Few cells were enclosed in this tissue which was either intimately applied to the bone and paramyloid or separated from one or the other by a single layer of spindle cells. In spite of its close application to paramyloid it usually failed to merge with it and remained conspicuous by its staining reaction, by its distinct tendency for irregular calcification and by the entrapped cells which had features of osteocytes. We are inclined to consider this apparently homogenous tissue to be of hyalin nature and a kind of "osteoid" with a tendency toward focal granular calcification. However, there were some areas which failed to reveal a sharp border between calcifying "osteoid" and calcifying layered paramyloid. This statement does not disagree either with the concept of the osteoid nature or the concept of the paramyloid nature of these tissues,

providing one accedes that the earliest stage of formation of primitive bone is hyaliniration of the intercellular substance of the connective tissue and that hyalin is somehow related to paramyloid.

The distribution of paramyloid differs from that of amyloid by its failure to occur in the liver, spleen and kidneys, *i.e.*, in organs which are usually involved in amyloidosis. The first case showed these organs to be free of amyloid and there was not the slightest clinical evidence of involvement of the parenchymatous organs in the second case.

2. The changes of the paramyloid bodies occurred both on their surface and within their interior. Almost all paramyloid aggregations were surrounded by monstrous giant cells, some of which were wrapped around the bodies in snake-like fashion. In contrast to the extensive lacunar resorption of amyloid described by some authors there was only moderate resorption noted in our first case in spite of the many giant cells. Here and there spindle-shaped reticulum cells were observed in shallow lacunae. Some of the giant cells contained large nuclei poor in chromatin, each with a nucleolus, while others had sickle-shaped hyperchromatic nuclei in an eosinophilic cytoplasm. The latter were probably degenerating cells. Freund[11] considered the possiblity that the giant cells originated from the endothelial cells of the many capillaries with which they appeared to be connected. We were led to believe that certain giant cells may be of this origin. A great number of giant cells displayed a peculiar breakdown of the cytoplasm. The cell membrane disappeared, the cytoplasm became spotty and cloudy, vacuoles became visible and droplets of homogenous material were liberated to combine with the paramyloid.

Many paramyloid accumulations displayed such extensive calcification that they should have been noted in the roentgenograms. Freund and others described the calcification of amyloid in bone marrow. The deposition of calcium salts proceeded irregularly in rather concentric layers from the surface toward the center, to form, finally, compact calcified masses as seen particularly in the vertebra. Fractures of such calcified paramyloid bodies were not uncommon (Fig. 7). Occasionally their fragments appeared united by connective tissue and sometimes by "osteoid" substance.

Degenerative changes of some of the paramyloid clumps in the vertebra were of interest because of a kind of dissolution initiated by the appearance of light spots with radiating lines in the calcified substance (Fig. 11). This disintegration proceeded to complete disappearance of the paramyloid. Identical changes were noted by Freund and were attributed by him to enzymatic action. Dissolution of amyloid in the "sago-spleen", liver, and other organs has been described.

3. Of interest is the relationship of the paramyloid to bone trabeculae, bone marrow and cartilage. The paramyloid masses began to form in the bone marrow, then crowded it out, causing the spongy bone trabeculae to be resorbed; the cortex became thinned and was perforated in places, the paramyloid extending into the periosteum. Microscopic examination of the sphenoid and rib failed to reveal the action of paramyloid upon the bone trabeculae since most of them were replaced by paramyloid and the newly-formed trabeculae were almost uni-

formly in a stage of deposition. Only the inner side of the cortices and the trabeculae of the vertebra offered the opportunity to observe resorption. The resorption was associated with a preponderance of spindle-shaped reticulum cells while mutlinucleated osteoclasts were distinctly scarce. Even in areas where the deposition of paramyloid appeared intimately connected with the bone, resorption by only small cells has been noted. The trabeculae of the vertebra in the vicinity of paramyloid bodies were in an inactive stage, *i.e.*, neither resorption nor deposition was noticeable.

The cortex of the sphenoid and rib showed very little periosteal bone deposition whereas the endosteal bone formation was remarkable in areas where, following more or less complete resorption of paramyloid, young connective tissue became active. This observation is in contradiction to those of some authors who stressed the lack of new bone production. Numerous paramyloid bodies appeared embedded in bone trabeculae.

Concerning the bone marrow cells, the outstanding feature in the sphenoid and rib was the abundance of hyperchromatic reticulum cells, free-lying giant cells, and a few fat cells in extremely vascular tissue containing much "paramyloid". Some plasma cells and lymphocytes were present and, in accord with other authors, must be regarded as an inflammatory reaction. Occasionally small arteries showed paramyloid alteration of the media and intima. This led not only to a remarkable reduction of the lumens but also to complete obstruction. Some of the occluded arteries were surrounded by giant cells. Occasionally the paramyloidosis also affected the adventitia and coalesced with adjacent paramyloid bodies. The larger arteries of the periosteum, however, displayed only proliferation of the intima.

The involvement of cartilage cells as seen in the vertebra presented an arresting feature not hitherto described. Paramyloid rings encircled the capsules of individual cartilage cells and extended outward and inward as well, thereby not only incorporating nearby cells into the paramyloid, but also filling in the cells (Fig. 10).

The outstanding involvement of the epifascial fat by the amyloid offered the opportunity to study the mode by which the amyloid altered fat tissue. Recently Pearson and associates[25] described "amyloid rings" around the fat cells and expressed the opinion that they were helpful in making a diagnosis from biopsy specimens. Peters[26] pointed out that fat cells first became enveloped by rings and shells of amyloid and then had undergone gradual atrophy. He came to the general conclusion that "the deposition of amyloid on cells rather than deposition in the interstices of mesenchymal tissue may be the starting point of amyloidosis." Our findings, however, prove that a narrow amyloid ring was first deposited upon the membrane of the fat cell without any interference with the shape of the cell (Figs. 8 and 9). Thereafter, droplets of amyloid collected between the fat cells and small connective tissue cells and a few lymphocytes appeared. The spotty increase of this intercellular amyloid compressed the fat cells so that they became deformed to narrow slits which were still outlined by sharply demarcated amyloid rings. The interstitial paramyloid exerted such

pressure upon the paramyloid rings that they broke down and their remains were visible in the paramyloid as darkly-stained arcuate lines. Only after this change did the cell membrane fade away and paramyloid advance toward the center of the fat cell, gradually filling it. The paramyloid in the fat cells occasionally contained a few connective tissue cells and lymphocytes.

4. The important questions concerning these two cases of tumor-like accumulation of paramyloid are: (a) Has the diagnosis of multiple myeloma been missed? (b) have "localized plasmocytomas" or (c) has an osteomyelitis or other chronic infective process been the underlying cause? or (d) is the "amyloid" of primary nature, *i.e.*, without detectable cause?

(a). To exclude multiple myeloma we must rely on the clinical, roentgenologic, hematologic and biochemical examinations. These were negative. Yet the evidence which showed most clearly that we were not dealing with a myelomatosis was the lack of characteristic myeloma cells. The presence of occasional plasma cells intermingling with lymphocytes and proliferating connective tissue cells among paramyloid bodies may, most likely, be attributed to a simple inflammatory reaction. In the amyloid formation in myeloma the myeloma cells diminish as amyloid deposition proceeds; yet in the amyloid-free bone marrow, myeloma cells persist. There was complete absence of plasma cells in the vertebra. We are not aware of any instance of spontaneous regression and cure of a myeloma, and, therefore, do not consider this case one of a theoretically healed myeloma.

(b). The second question has been answered in the foregoing discussion, since we believe that there is insufficient proof for regarding the so-called "solitary or localized plasmocytoma" as a clinical entity. Several recorded cases of "solitary" myeloma were based on the period of good health (up to nine years) following the treatment of the lesion (Coley,[8] Stewart and Taylor,[32] and others). Willis[38] observed a "solitary plasmocytoma" of bone which had probably been present for about twenty years. However, Bayrd and Heck[4] claim that the solitary and multiple myelomas "are probably all a phase in the same disease in which the plasma cell leukemias represent one extreme, and the solitary myeloma the other." Lichtenstein and Jaffe[20] took a similar point of view.

(c). The clinical, laboratory and microscopic findings in the first case were not suggestive of a long-standing infective disease.

(d). We are not able to place the first case in any one of the preceding groups, whereas the second case is possibly of traumatic origin.

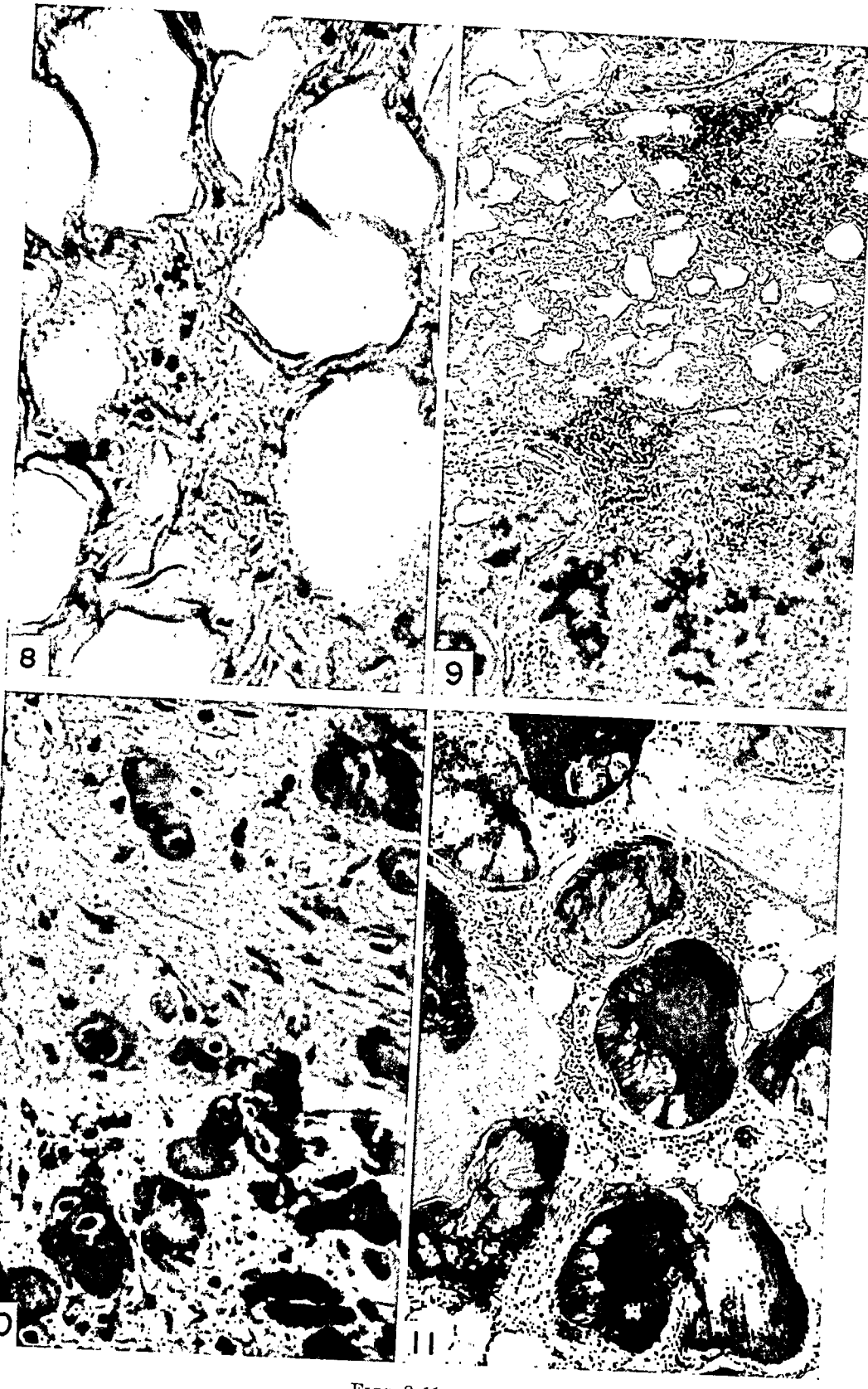
5. The most difficult problem is to find an acceptable explanation of the patho-

FIG. 8. Case 2. Paramyloid involving fat. Note paramyloid rings along cell-membranes, paramyloid droplets, and remains of broken paramyloid rings between compressed fat cells. H and E. × 450.

FIG. 9. Case 2. Partially calcified paramyloid in fat tissue and dense lymphocytic infiltration within fat. Note distortion of fat cells. H and E. × 300.

FIG. 10. Case 1. Section of intervertebral disc of ninth vertebra. Area of collapse of the vertebral cartilage with invasion by young connective tissue. Dislocated cells of disc embedded in paramyloid. H and E. × 450.

FIG. 11. Case 1. Section of vertebra showing heavily calcified paramyloid bodies with thready disintegration. They are in intimate relation to less-calcified bone trabeculae. Note complete absence of plasma cells. H and E. × 200.



FIGS. 8–11

genesis of local formation of atypical amyloid. The origin of atypical amyloidosis without known cause is obscure, but many attempts have been made to determine the cause of the variety of atypical amyloidosis which is related to the distinctive systemic disease, multiple myeloma.

It has been emphasized that paraproteinemia, *i.e.*, presence of one or more abnormal proteins (globulins), rather than hyperproteinemia, is one of the features of multiple myeloma. In accord with this assumption, some authors (Steinmann[31] and Apitz[7]) correlated the concurrent plasmacytosis with the production of the atypical amyloid, claiming that myeloma cells were active in the formation of abnormal proteins. Be that as it may, the precipitation of this or other abnormal proteins may lead to the deposition of systemically distributed paramyloid.

However, in order to understand the local deposition of paramyloid, we may assume that a local lesion, such as inflammation or trauma, may cause not only a derangement of the exchange of protein between the cells and the plasma but also the appearance of abnormal proteins. These precipitated and calcified proteins incite a foreign-body reaction.

As to the question, has the paramyloid been deposited inter- or intracellularly, our observations indicate that both modes occur at the same time and the findings of Whipple and Madden[37] proved that there is a protein flow from cell to plasma or from plasma to cell, and that flow may occur in both directions at the same time. We noted that various cells of the bone marrow had undergone alterations of the cytoplasm which led to gradual disappearance of their nuclei. The altered cytoplasm of such cells fused together and formed paramyloid bodies. Similarly, small globules of paramyloid formed in the reticulum of the bone marrow, increased in number and coalesced. These bodies appeared spotty and contained pyknotic nuclei of destroyed cells. Like other authors, we also noted paramyloid within giant cells. Though we did not observe a noteworthy resorption of paramyloid by giant cells in spite of their very great number, we do not reject the idea that some of the paramyloid in giant cells had been ingested. Our findings, however, showed very clearly that the cytoplasm of many giant cells became transformed into paramyloid.

SUMMARY

Two cases of solitary tumor-like accumulation of atypical amyloid (paramyloid) have been reported. The first case displayed paramyloid in the sphenoid bone, the ninth dorsal vertebra and the left ninth rib without a detectable cause. The second case showed paramyloid in a movable nodule in the soft tissue of the left thigh was probably the result of local trauma.

The term "paramyloid" signifies the atypical distribution and the variable staining reaction of a hyalin-like material.

Paramyloid, which seems to be related to amyloid and hyalin, may undergo calcification and may then become fractured. It may be resorbed by giant cells or be dissolved by an internal "enzymatic" process.

The appositional growth of atypical amyloid causes the resorption of bone.

Cartilage cells and fat cells are affected by paramyloid in a characteristic form. Paramyloid is formed in the cell, on the cell membrane, and in intercellular spaces.

Acknowledgment. We wish to thank Dr. L. H. Kretchmar and Dr. W. A. D. Anderson for permission to use Case 2.

REFERENCES

1. Apitz, K.: Die Paraproteinosen. Virchows Arch. f. path. Anat., **306:** 631–699, 1940.
2. Askanazy, M.: Das Knochenmark. Henke u. Lubarsch' Handbuch d. spez. path. Anat. und Hist., Berlin: Bd. I/II, 774–1014, 1927.
3. Atkinson, F. R. B.: Multiple myelomata. M. Press, **195:** 312–317, 327–333, 1937.
4. Bayrd, E. D., and Heck, F. J.: Multiple myeloma. J. A. M. A., **133:** 147–157, 1947.
5. Von Bonsdorff, B., Groth, H., and Pakalen, T.: On the presence of a high-molecular crystallizable protein in blood serum in myeloma. Folia haemat., **59:** 184–208, 1938.
6. Brachetto-Brien, D., Bado, J. L., and Cagnoli, H.: Solitary amyloid tumor of bone with generalized amyloidosis; report of case involving femur and review of literature. Soc. Argent. de anat. norm. y path., **7:** 182–208, 1945.
7. Bürgi, U.: Über einen Fall von solitärem Amyloidtumor des Scheitelbeines. Frankf. Ztschr. f. Path., **60:** 410–428, 1937.
8. Coley, W. B.: Multiple myeloma. Ann. Surg., **93:** 77–89, 1931.
9. Dahlin, D. C.: Primary amyloidosis with report of six cases. Am. J. Path., **25:** 105–125, 1949.
10. Duenner, M.: Plasmocytom des Manubrium sterni mit lokaler Amyloidbildung. Schweiz. med. Wchnschr., **76:** 1109–1110, 1946.
11. Freund, E.: Über diffuses Myelom mit Amyloidtumoren. Frankf. Ztschr. f. Path., **40:** 400–416, 1930.
12. Gerber, I. E.: Amyloidosis of the bone marrow. Arch. Path., **17:** 620–630, 1934.
13. Gordon, M.: Is rheumatism a virus disease? Lancet, **254:** 697–701, 1948.
14. Hass, G. M., Huntington, R., and Krumdieck, N.: Amyloid III. The properties of amyloid deposits occurring in several species under diverse conditions. Arch. Path., **35:** 226–241, 1943.
15. Hedren, G.: Ein Amyloidtumor des Knochenmarks. Ztschr. f. klin. Med., **63:** 212–219, 1907.
16. Hildebrand, O.: Über Corpora amylacea und lok. Amyloid in einem endostalen Sarkom des Brustbeines. Virchows Arch. f. path. Anat., **140:** 249–271, 1895.
17. Iverson, L., and Morrison, A. B.: Primary systemic amyloidosis. Arch. Path., **45:** 1–20, 1948.
18. King, L. S.: Atypical amyloid disease, with observations on a new silver stain for amyloid. Am. J. Path., **24:** 1095–1117, 1948.
19. Koletzky, S., and Stecher, R. M.: Primary systemic amyloidosis: involvement of cardiac valves, joints and bones, with pathologic fracture of the femur. Arch. Path., **27:** 267–288, 1939.
20. Lichtenstein, L., and Jaffe, H. L.: Multiple myeloma. Arch. Path., **44:** 207–246, 1947.
21. Loeschcke, H.: Vorstellungen über das Wesen von Hyalin und Amyloid auf Grund von serologischen Versuchen. Beitr. z. path. Anat. u. z. allg. Path., **77:** 231–239, 1929.
22. Lubarsch, O.: Zur Kenntnis ungewöhnlicher Amyloidablagerungen. Virchows Arch. f. path. Anat., **271:** 867–889, 1929.
23. Magnus-Levy, A.: Multiple Myeloma VII. Euglobulinämie. Zur Klinik und Pathologie von Amyloidosis. Ztschr. f. klin. Med., **126:** 62–111, 1933.
24. Mandl, J.: Über lokales Amyloid in Bereiche der Brustwirbelsäule. Virchows Arch. f. path. Anat., **253:** 639–655, 1924.
25. Pearson, B., Rice, M. M., and Dickens, K. La. V.: Primary systemic amyloidosis. Arch. Path., **32:** 1–10, 1941.
26. Peters, J. T.: Epicellular and pericellular depositions of amyloid as the starting point of amyloidosis. Arch. Path., **35:** 832–835, 1943.
27. Picchini, L., and Fabris, A.: Sulle paramiloidosi. Arch. per le sc. med., **54:** 551–569, 1930.
28. Randerath, E.: Zur pathologischen Anatomie der sogenannten Amyloidnephrose. Virchows Arch. f. path. Anat., **314:** 388–459, 1947.
29. Rosenblum, A. H., and Kirschbaum, J. D.: Multiple myelomas with tumor-like amyloidosis. J. A. M. A., **106:** 988–991, 1936.
30. Schmidt, H.: Über lokale Amyloidosis im Bereiche des Oberkiefers und der Wirbelsäule. Inaug. Diss., Zurich, 1919.

31. STEINMANN, B.: Über azurophile, stäbchenförmige Einschlüsse in den Zellen eines multiplen Myeloms. Deutsches Arch. f. klin. Med., **185:** 49–61, 1939.
32. STEWART, M. J., AND TAYLOR, A. L.: Observation on solitary plasmocytoma. J. Path. and Bact., **35:** 541–547, 1932.
33. STRAUSS, A.: Über Paramyloidose. (Mit Wiedergabe eines eigenartigen Falles von ausgedehntem Binde-und Fettgewebsamyloid) Virchows Arch. f. path. Anat., **291:** 219–236, 1933.
34. TEILUM, G.: Allergic hyperglobulinosis and hyalinosis (paramyloidosis) in the reticuloendothelial system in Boeck's sarcoid and other conditions. Am. J. Path., **24:** 389–409 1948.
35. TRASOFF, A., SCHNEEBERG, N., AND SCARF, M.: Recovery from multiple rheumatoid arthritis complicated by amyloidosis in a child; report of a case and review of literature. Arch. Int. Med., **74:** 4–10, 1944.
36. VOGELER, K.: Amyloidtumor des Schädels im Röntgenbilde. Arch. f. klin. Chir., **180:** 37–39, 1934.
37. WHIPPLE, G. H., AND MADDEN, S. C.: Hemoglobin, plasma protein and cell protein—their interchange and construction in emergencies. Medicine., **23:** 215–224, 1944.
38. WILLIS, R. A.: Solitary plasmocytoma of bone. J. Path. and Bact., **53:** 77–85, 1941.
39. YEOMAN, W., AND WILSON, I. V.: Rheumatoid arthritis and amyloid disease. Brit. M. J., **2:** 483–485, 1947.

AN IMPROVED METHOD OF COUNTING BLOOD PLATELETS

And Its Evaluation in Blood of Rabbits*

GEORGE J. SCHEFF, M.D., and PAUL H. RALPH, Ph.D.

From the Division of Medical Research, Department of Medicine, Ohio State University, Columbus, Ohio

The counting of blood platelets is, in spite of the multiplicity of the proposed methods, one of the most unreliable procedures of clinical hematology. The usual methods of counting blood platelets yield results which vary greatly in comparison with those of the other formed elements of the blood.

A new method would seem justified only if it is more accurate. Our direct dark-field technic of counting platelets, a modification of the method of Vilariño and Pimentel,[7] is presented in the belief that it is more accurate than other methods in use.

MATERIALS AND APPARATUS

1. A modified Aynaud's fluid was used both as fixative and diluent. It was prepared fresh every three days by mixing 10 ml. 38 per cent sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 5 ml. 12 per cent sodium chloride, and 10 ml. 37 per cent neutral formaldehyde solution, and adding distilled water up to 100 ml. in a volumetric flask. The constituents of this fluid were kept as stock solutions (the formaldehyde being neutralized with marble chips).

2. Two types of centrifuge tubes were employed. The first tube, X, was made by cutting 4-inch pieces of 8-mm. bore glass tubing, and sealing off one end. They were marked at the top of the meniscus to indicate levels of 0.5 ml. and 1 ml. of fluid. They were also marked at a distance of 7 cm. from the bottom of the tube. The second tube, Y, which served the purpose of collecting and diluting the pooled platelets was a standard Kahn tube marked at 2.5 ml. and 5.0 ml.

3. A Sorvall Type-SP angle centrifuge was used for fractional centrifugation, but any other angle centrifuge permitting regulation of speed should be satisfactory.

4. A Petroff-Hausser bacterial counting chamber with a hemocytometer cover glass.

5. A Spencer binocular microscope was used with the following accessories: (a) a bispheric dark-field condenser (Spencer) of sufficient focal length to illuminate specimens in the Petroff-Hausser counting chamber; (b) a pair of wide-field 18 × eyepieces; (c) a 4 mm. dry objective, NA 0.66, magnification 44 ×; (d) a special funnel stop which was made by shortening the tube and widening the opening of a regular funnel stop used in an oil-immersion objective; (e) a Spencer microscope lamp with 200-watt, 120-volt projection bulb, focusing condenser and heat-absorbing glass filter.

6. Capillary pipets, syringes (2 ml. Yale Lok) and needles for drawing blood.

PROCEDURE

1. Aynaud's fluid was drawn into a syringe which was adjusted to the 1 ml. mark, and a new dry needle was then attached.

2. One ml. of blood was drawn (from the central artery of the rabbit's ear, or from the antecubital vein of man) by inserting the needle in the well-dilated blood vessel and waiting until the inflowing blood pushes the plunger of the

* This work was aided by a grant from the Ella Sachs Plotz Foundation.
Received for publication, June 30, 1949.

syringe to the 2 ml. mark. Then the needle was quickly withdrawn from the blood vessel.* A large bubble of air was permitted to enter the syringe containing the sample and the syringe was then inverted twenty times. After removing the needle from the syringe, the blood was expelled into a Kahn tube and the tube was tightly stoppered with a clean cork stopper.

3. The Kahn tube containing the mixture of blood was inverted ten times. Then, the mixture was withdrawn with a clean capillary pipet and was introduced into the special centrifuge tube, X, to the 0.5 ml. mark in the case of rabbit blood, or to the 1.0 ml. mark in the case of human blood. Care should be taken not to include air bubbles. The tube was filled up to the 7 cm. mark with the diluting fluid and the contents were mixed by closing the open end with the tip of a finger.

4. The tube was placed in a centrifuge which was allowed to run for five seconds after being set at highest speed; the rheostat was then regulated to 700-750 RPM. After five minutes of centrifugation the red cells should be loosely packed.

TABLE 1

PLATELETS RECOVERED BY FRACTIONAL CENTRIFUGATION

	CONSECUTIVE WASHINGS					TOTAL
	1	2	3	4	5	
Blood sample #1	790,000	69,000	12,000	3000	500	874,500
Blood sample #2	803,000	73,500	12,500	1500	0	890,500
Average	796,500	71,250	12,250	2250	250	882,500
Per cent of the total	90.3	8.1	1.4	0.2	0.03	

5. The supernatant fluid (containing the platelets) was withdrawn with a capillary pipet, and was placed in a clean Kahn tube, Y, marked at 5 ml.

6. The tube, X, was refilled with Aynaud's fluid to the 7 cm. mark. The tube was inverted until blood and diluent were well mixed. Step 4 was then repeated but for only three minutes. The supernatant fluid was again withdrawn and added to that previously collected in tube, Y.

7. Step 6 was repeated, filling only to the 1.0 ml. mark and centrifuging in the same manner as before for two minutes only.

8. The pooled supernatant fluids in tube, Y, containing the platelets were made up to 5 ml. by adding the necessary amount of diluent. The tube was stoppered and placed on a shaker (200 strokes per minute) for two minutes.

9. A drop of suspension was placed on the stage of a counting chamber with clean capillary pipet and a cover slip was immediately dropped over it. From three to five minutes should be allowed for convection to diminish.

10. The platelets were counted in five large squares (*i.e.*, in 80 small squares).

* If the direct use of a syringe containing Aynaud's fluid is objectionable, the blood may be drawn with a dry 5 ml. syringe which has a hole drilled at its plunger-end. This permits the air to enter the syringe. The blood may be withdrawn from it into the prescribed 2 ml. syringe from the needle-end in the usual manner.

The number of platelets in 1 cu. mm. of blood was calculated by multiplying the average number of platelets in one small square by 400 (base), by 50 (depth), and by 20 (dilution) in the case of 0.5 ml. of mixture containing equal parts of blood and Aynaud's fluid, or by 10 in the case of 1.0 ml. of the mixture. In practice the resultant number of platelets in five large squares was divided by 2 (if the dilution was 20 times) and multiplied by 10,000, or the division by 2 was omitted if the dilution was only 10 times.

TABLE 2

PLATELET COUNTS IN 18 RABBITS

ANIMAL NUMBER	NUMBER OF PLATELETS PER CU. MM. BLOOD (Each figure is the mean of three counts)				AVERAGE NUMBER OF PLATELETS
	First Sample		Second Sample		
	First Half (a)	Second Half (b)	First Half (c)	Second Half (d)	
50	593,333	560,000	550,000	550,000	563,333
51	660,000	665,000	668,333	671,666	666,249
52	666,000	669,333	632,333	—	655,888
53	872,333	840,000	905,666	882,666	875,166
54	611,666	625,000	639,000	637,000	628,166
55	619,000	600,666	595,000	628,333	610,749
56	790,666	782,666	824,000	800,333	799,416
57	619,666	613,000	623,000	638,333	623,499
58	566,000	559,666	565,000	571,333	565,499
59	897,666	887,000	888,333	903,333	894,083
2	883,333	—	876,666	—	879,999
19	845,000	—	890,000	—	867,500
20	1,033,000	—	1,026,666	—	1,029,833
20*	906,666	—	940,000	—	923,333
21	956,666	—	880,000	—	918,333
21*	911,666	—	886,666	—	899,166
30	846,000	—	888,333	—	867,166
42	905,000	—	923,333	—	914,166
43	905,000	—	906,666	—	905,833
46	791,666	—	816,666	—	804,166

* Samples were taken on different days.

RESULTS

In establishing the efficacy of this method for research purposes, numerous platelet counts have been performed during 1944–1946 on rabbits each weighing six to seven pounds. A few counts on man were also included in this study for general orientation.

Table 1 shows the platelet recovery after multiple fractional centrifugations in two samples of blood. It confirms previous observation that a single centrifugation is inadequate to obtain the total number of platelets. Repeated centrifugations and washings are necessary to obtain a value approaching the total number of platelets. Since, however, the absolute number of platelets recovered after each successive centrifugation decreases asymptotically, it is evident that

this tedious procedure can be limited by proper standardization. In our experience three centrifugations were sufficient to obtain maximal counts (99.8 per cent) of platelets within the limits of experimental error.

TABLE 3

THE STABILITY OF PLATELETS IN AYNAUD'S FLUID DURING STORAGE IN THE REFRIGERATOR

SAMPLE	COUNT AT BEGINNING	3 HR.	COUNTS AFTER STORAGE OF		20 HR.
			5 Hr.	8 Hr.	
#1	815,000		810,000		785,000
#2	608,000	615,000			560,000
#3	749,000		745,000		685,000
#4	865,000		866,000	855,000	810,000

Table 2 illustrates the variability of the method (a) on a single sample after it has been subdivided into two parts, and (b) on two samples taken from the left and right ears, at short intervals, from the same animal. The latter counts obviously include the error of sampling, while the counts performed on a single sample exclude it.

TABLE 4

VARIABILITY OF REPEATED PLATELET COUNTS IN INDIVIDUAL RABBITS

ANIMAL NUMBER	NUMBER OF COUNTS*	EXTREMES ($\times 10^3$)	MEAN	σ	COEFFICIENTS OF VARIATION
19	8	848–1083	947,750	68,372	7.2
20	5	857–1033	928,400	65,167	7.0
21	3	912–980	949,700	41,189	4.3
22	3	892–1040	943,000	84,042	8.9
23	3	977–1100	1,021,700	68,061	6.6
42	3	905–933	916,000	14,933	1.6
43	3	905–960	926,666	29,297	3.2
46	3	792–797	794,000	2,646	0.3
Total counts	31				
Averages		792–1100	931,420	73,686	7.9

* Each count was made on a different day.

In the first ten animals the difference between the two parts of a single sample did not exceed 33,333, but was usually a great deal less. Taking the first halves of the two samples (a and c) from the same animals as one series (20 counts), and the second halves (b and d) as another series (19 counts), the following results were obtained:

SERIES	AVERAGE	DIFFERENCE (D)	STAND. DEVIATION (σ)	STAND. ERROR (E)	E_D	$\frac{D}{E_D}$
First half	689,349	648	123,065	27,518	38,617	0.01
Second half	688,701		118,096	27,093		

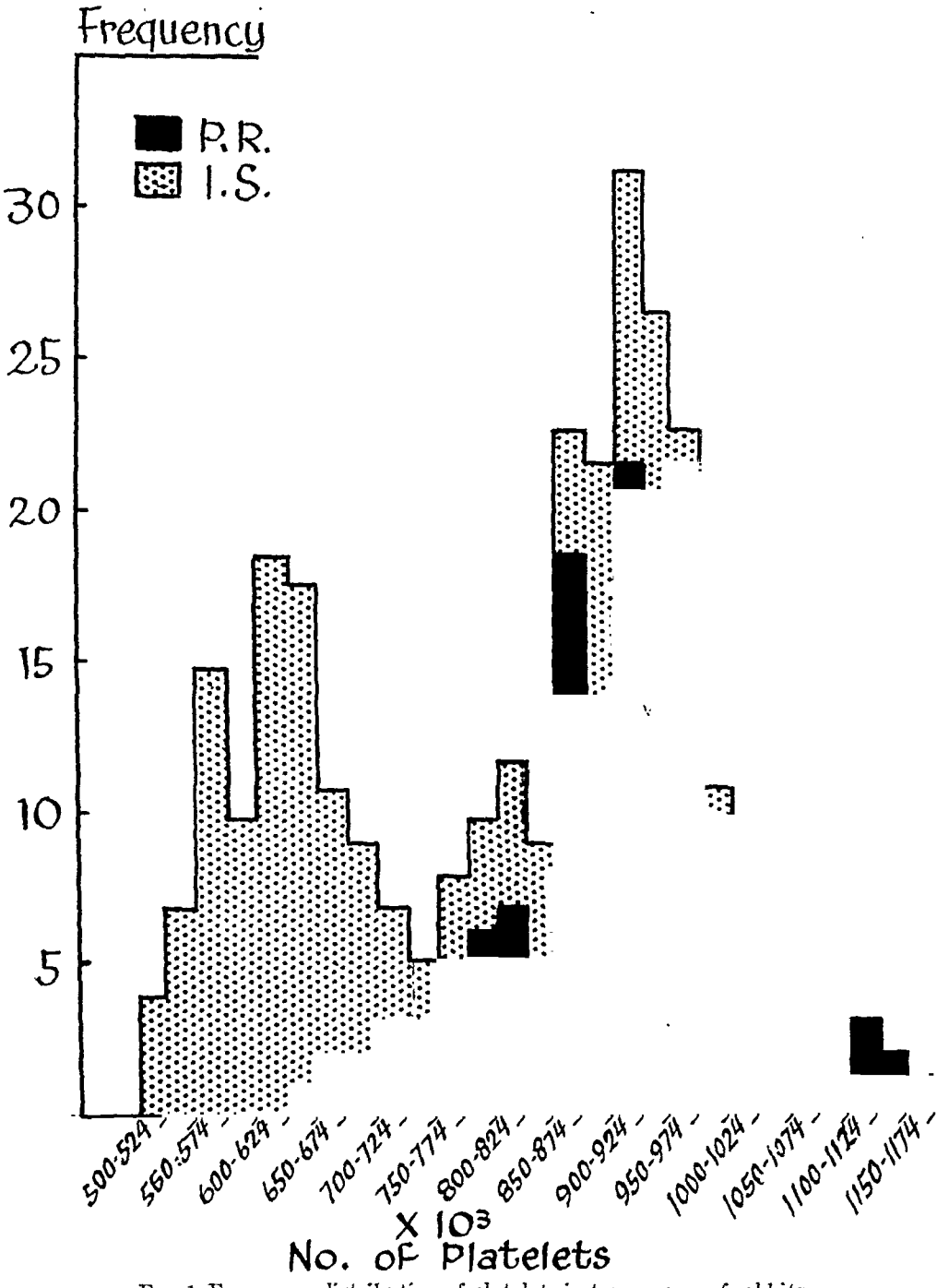


FIG. 1. Frequency distribution of platelets in two groups of rabbits

Then, comparing the first halves (a and c) of the two samples of all 18 animals, the difference between the platelet figures of these two columns was found

to be somewhat larger but did not exceed 76,666. Analyzing these two groups further (both with 20 counts), the results were as follows:

SERIES	AVERAGE	DIFFERENCE (D)	STANDARD DEVIATION (σ)	STAND. ERROR (E)	E_D	$\frac{D}{E_D}$
a	793,716	2567	142,388	31,839	45,896	0.05
c	796,283		147,835	33.057		

Apparently sampling does introduce some error but the difference is negligible. Accepting Fisher's standard, the variation in both series is insignificant.

TABLE 5

VARIABILITY OF SINGLE PLATELET COUNTS MADE BY TWO OBSERVERS, EACH ON 18 RABBITS

OBSERVER	NUMBER OF ANIMALS	EXTREMES	MEAN	σ	COEFFICIENTS OF VARIATION
P.H.R.	18	650,000–1,130,000	877,963	112,291	13.9
I.M.S.	18	537,000– 898,000	652,338	117,023	17.9
Averages		537,000–1,130,000	768,150	154,719	20.1

Table 3 demonstrates the stability of platelets in Aynaud's fluid during storage in the refrigerator. From 13 such counts on four samples one may conclude that up to eight hours the platelets do not deteriorate, especially if they are separated from the blood soon after withdrawal. Their decline even after twenty hours is slight.

TABLE 6

RANDOM PLATELET COUNTS IN 13 PERSONS

NAME	NUMBER OF PLATELETS	NAME	NUMBER OF PLATELETS
E.	620,000	Be.	590,000
B.	620,000	Br.	600,000
H.	560,000	Da.	660,000
D.	550,000	L.	630,000
Ha.	670,000	G.	630,000
		S.	560,000

Table 4 contains the results of from three to eight base-line counts which were done for each of eight rabbits. The coefficients of variation for each rabbit oscillated between 0.3 and 8.9 and was only 7.9 for all the counts (31). Therefore, contrary to the general opinion that fluctuations in the platelet levels of rabbits are large and unpredictable, our observations indicate that the variation is relatively small. It would seem probable that if all other variables (season, breed, age and sex) were also taken into account, the differences were even more limited.

Figure 1 indicates the frequency distribution of 305 platelet counts made by

two independent observers (P.H.R. and I.M.S.) on two different groups of rabbits. The dotted bars refer to the results obtained in a heterogeneous group of animals obtained from various sources, and the solid bars refer to the results obtained in a more homogeneous group from stock bred at the University Farm of Ohio State University. In the former there are two maximums, whereas in the latter the single maximum coincides with one of the peaks observed in the heterogeneous group. It is believed, therefore, that racial differences rather than personal errors may account for the discrepancy in the two groups of animals. Such a conclusion awaits the study of a larger series of animals.

The variability of a rabbit population of 36 animals with a single count on each gave the results shown in Table 5. Evidently the high coefficient of variation (C. V. = 20.1) indicates that the platelet fluctuation in different animals is much larger than was found previously in daily changes of single animals (C. V. = 7.3).

Table 6 gives the platelet counts of ten healthy medical students and of one of the authors. The bloods were drawn before breakfast.

The variation within this limited group was very small. This is perhaps a good indication that the number of platelets in man is much more constant than is commonly believed. In a few instances the effect of food intake (breakfast) was also studied but apparently such minor stimulation does not cause significant changes in the count.

DISCUSSION

The two major difficulties inherent in platelet enumeration are (1) incomplete preservation of all individual units, and (2) unreliable microscopic identification of true platelets. Unlike the other cellular blood constituents, the platelets are extremely difficult to preserve outside the intact circulation even in the presence of anticoagulants. Mild fixation appears to be the only effective way to protect them from early destruction. By drawing circulatory blood directly into Aynaud's fluid, as above indicated, instead of using capillary blood, one is assured of a more uniform sampling of the platelets, and also of their immediate fixation. Once fixed, the platelets survive considerable handling.

Separation of the platelets from other blood cells was best achieved by standardized fractional centrifugation. As Vilariño and Pimentel[7] have demonstrated, this seems to be necessary because of the rapid sedimentation of the platelets. However, Copley and Robb[2] have shown, that, in case of proper standardization, less than five centrifugations are sufficient to approximate the maximal number. Under the conditions we have outlined, three centrifugations yield 99.8 per cent of the platelets found after five centrifugations.

The basic improvement in the present technic is the adaptation of the direct counting method to dark-field observation. The superiority of direct counting, versus indirect, has been recognized by other investigators. It eliminates errors resulting from uneven distribution of red cells and platelets in methods based on the relative proportion of one of these elements to the other. Thus, preliminary dark-field observations revealed many red cell shadows (ghost cells) which

would have escaped observation in a bright field. This does not necessarily affect the red cell count *per se* but magnifies the error in platelet counting by indirect methods. Most of all, the dark-field observation offers an easy method of identification. It makes staining unnecessary and eliminates the possibility of confusing debris and stain-crystals with platelets. Furthermore, the Petroff-Hausser counting chamber used in this method is only one-fifth (0.02 mm.) of the depth of the regular Neubauer counting chamber (0.1 mm.) commonly used in bright-field counting procedures. Since normally platelets are 2–4 μ in diameter, the error of missing them because of their being out of focus is much less. The error due to convection is also reduced.

With our technic we found higher concentrations of platelets in the blood of rabbits and man than those reported in the literature.[6] Ecker and Rees,[4] using their own method, in 1922 reported an average platelet value of 557,000 for eight normal rabbits with a minimum count of 368,000 and a maximum of 853,000 platelets. Scarborough[5] in 1926 compiled the platelet counts for rabbits from the results of ten different investigators, most of whom used different methods and studied only a few animals. These averaged 540,000 platelets per cu. mm. of blood with limits of 200,000 and 1,000,000. Dudgeon and Goadby[3] in 1931 determined platelet values for three normal rabbits of 503,400, 489,000, and 1,009,200 respectively. The most complete study has been made by Casey and Rosahn[1] in 1932 using Casey's method. They found in 991 counts made on 148 rabbits, an average of 532,645 platelets, with extremes of 170,000 and 1,120,000 (C. V. = 23.5).

Our observations which include 305 counts on 36 animals gave an average of 765,150 platelets for the total rabbit population, the extremes ranging from 537,000 to 1,130,000 (C. V. = 20.1). An incidental finding in the frequency distribution curve of two peaks strongly suggests the possibility of racial differences in the platelet count of rabbits.

The ten human counts averaged 607,000 platelets per cu. mm. of blood, which is somewhat high considering that 250,000 to 500,000 platelets have been accepted as the normal range. There are, however, a few reports with similarly high counts on record.[6] Presently no better explanation can be offered for our high platelet counts than the improved technic. This is supported by the relative constancy of the repeated platelet counts of the individuals. It seems, therefore, that the platelets, as far as counting is concerned, can be determined with the same degree of accuracy as any of the other blood cell elements.

CONCLUSION

An improved platelet counting technic is offered which is particularly useful for research. The method is considered to be more accurate and reliable than those in present use.

REFERENCES

1. Casey, A. E., and Rosahn, P. D.: Studies on blood cytology of rabbits; blood platelet counts on healthy male rabbits. J. Exper. Med., **56**: 841–851, 1932.

2. Copley, A. L., and Robb, T. P.: Studies on platelets. I. Method of Vilariño and Pimentel and new direct method of counting blood platelets, Am. J. Clin. Path., **12:** 362–371, 1942.
3. Dudgeon, L. S., and Goadby, H. K.: The examination of tissues and some observations on blood platelets of rabbits. J. Hyg., **31:** 247–256, 1931.
4. Ecker, E. E., and Rees, H. M.: Effect of hemorrhage on complement of blood. Compilation of published data. J. Infect. Dis., **31:** 361–365, 1922.
5. Scarborough, R. A.: The blood picture of normal laboratory animals. Yale J. Biol. and Med., **3:** 63–80, 1930.
6. Tocantins, L. M.: The mammalian blood platelet in health and disease. Medicine, **17:** 155–260, 1938.
7. Vilariño, M., and Pimentel, J. V.: Über Thrombocytenzählungen. Klin. Wchnschr., **18:** 1253–1256, 1939.

EFFECT OF SPLENIC EXTRACTS ON BLOOD PLATELET COUNTS IN RABBITS*

GEORGE J. SCHEFF, M.D., AND IRENE M. SCHEFF, M.D.

From the Division of Medical Research, Department of Medicine Ohio State University, Columbus, Ohio

The premise for the existence of a platelet-reducing agent (thrombocytopen) is based on the concept of Frank[4] that "the spleen exerts a myelotoxic and platelet-repressing effect." Since Frank's time, various methods of extraction have been employed to isolate the active principle from the spleen, but results obtained have been controversial. At present the contending forces are about equally divided, and there must be some reason for this discrepancy.

One of the chief handicaps in solving this problem has undoubtedly been the unreliability of the methods of counting platelets. Added to this is the fact that, aside from the spleen, a variety of biologic, chemical and nervous factors also produce similar depression of the platelet count, and these factors must be ruled out. In this paper we present our findings and correlate them with the observations of other investigators.

METHODS

The spleens for this work were obtained directly from the operating room. The extracts were prepared according to two different procedures. One method adopted the pattern of Troland and Lee[22] and their followers by using an acetone extract. In this method the spleen was finely ground and extracted with two to five volumes by weight of acetone at 4 C. for three to six weeks; thus, the time of extraction was varied. After storage, the mixture was filtered and the solvent was evaporated under reduced pressure with the production of a brownish gummy residue. The residue was resuspended in distilled water or saline (0.5–1.0 ml. per gram of tissue) and filtered through filter paper or occasionally through a Seitz filter. The extracts were tested immediately after their preparation. The other method followed the pattern of Deutsch *et al.*[3] by using sodium sulfate (Na_2SO_4) for the preparation of concentrated protein-free aqueous tissue extracts. Apparently this method had not previously been applied in making extractions of the spleen. The splenic tissue, freed from fibrous matter, was finely ground in a meat grinder. To the resultant mash, warmed to 32 C. in a water bath, 40 Gm. anhydrous Na_2SO_4 was added per 100 Gm. of tissue, stirred for five hours and kept in a tightly-covered container at 32 C. overnight. The exuded liquid was poured off and the remaining moisture expressed by a hand press. All these manipulations were carried out at 32 C. to prevent crystallization of the salts. Then the juices were collected and filtered while still warm and placed in the cold at 0 C. The decahydrate crystals of Na_2SO_4 which separated out were removed on a filter funnel at low temperature. The filtrate contained the active principle. Owing to certain inevitable manipulative losses, the actual yield of

* Aided by the Comly Research Fund.
Received for publication, June 30, 1949.

TABLE 1

PLATELET COUNTS (COUNT × 10^3) IN RABBITS AFTER ADMINISTRATION OF ACETONE EXTRACTS PREPARED FROM SPLEENS OF PATIENTS WITH THROMBOCYTOPENIC PURPURA

Patient / Date		Di 8/45	Ri 7/46	Kr 7/46	Du 7/46		Cr 10/46	Ca 12/46	B 3/47	K 4/47
Spleen	Weight (Gm.)	—	55	158	172		185	—	145	180
	Equivalent injected (Gm.)	10	10	10	10	20	(1) 10 (2) 10* →	10	10	10
Rabbit No.		I	IV	V	VIII	IX	XII	XVII	XXI	XXVI
	hr.									
Reading before injection		610	715	560	685	740	615	575	540	675
Readings after injection	½			518			568		466	665
	¾		638							
	1			468 (−16%)				519 (−9%)	371 (−31%)	648 (−4%)
	1½		612 (−14%)		645 (−5%)	675 (−8%)	535 (−13%)			
	2	540 (−11%)		525						659
	3		696				590		424	
	6	580								
	24	645	754	582	705	690	→ 624	555	548	
	25½						572 (−8%)			
	27						579			
	48		705		698	728	602		560	
	72							588	554	
	96			575	715	721				
	120								523	

* Second injection was administered after the 24-hr. sample was taken.

filtrate was somewhat less than theoretically expected. This was no real disadvantage since the active principle in the filtrate was about three times as concentrated as that in the original tissue. The fluid was slightly colored, neutral and completely protein-free; it contained 4 per cent Na_2SO_4. The amount of fluid usually injected corresponded to about 10–20 Gm. of splenic tissue.

The platelet counts were performed by a recently developed method.[16] First, the base-line count was established for each rabbit (weight, 5–6 lb.) to be tested by drawing blood from the central artery of one ear. Then, the freshly prepared extracts were injected into the marginal vein of the other ear. No untoward effects of such injection were observed at any time. The platelet counts were repeated at different intervals and were concurrently studied with the controls.

RESULTS

In view of the controversial findings of previous investigators, which will be discussed later, it seemed obligatory to repeat the original observation of Troland and Lee.[22] Table 1 contains the pertinent data which were obtained with splenic extracts prepared by the acetone-extraction method of these authors. The spleens were obtained from eight patients with well-established thrombocytopenic purpura. These patients were thoroughly studied on the medical services of the University Hospital (Dr. C. A. Doan and Dr. B. K. Wiseman). The case reports are omitted.

Within the first two hours after the injection, there appeared to be a slight reduction (av. 13 per cent) in the number of platelets which, however, never lasted more than five or six hours. The extent and duration of this effect was so limited that it did not approach the claim made by Troland and Lee[22] and their followers. Moolten[9] noted an initial increase in number of blood platelets shortly after injection, which our experiments failed to confirm. Two rabbits (VIII, IX) were injected with splenic extract from patient Du; in spite of the double dose received by one of them (IX), there was no difference in the response of the two animals. In rabbit XII the same amount of extract was injected on two successive days; the effect was lessened rather than increased. Repeated daily platelet counts did not reveal any significant change up to 120 hours. It would seem, therefore, that whatever decrease of platelets occurred following the injection, this was not caused by a specific splenic substance but rather by the physical nature of the injected material.

Table 2 gives data concerning the control experiments which were conducted concurrently with experiments with the splenic extracts. It is readily seen that the changes following the injection of saline and isotonic glucose solution (5 per cent) are negligible and are within the limits of experimental error. It also illustrates the important point, emphasized in a previous paper,[16] that in rabbits the platelet count varies from animal to animal, but is fairly constant in the same animal in the course of repeated sampling. This is not in accord with the experience of Uihlein[23] who abandoned the use of rabbits and worked with rats because of the inconsistencies of platelet counts in rabbits. In addition to the foregoing procedures, various inorganic and organic colloidal suspensions were used. India ink in saline suspension brought about an extreme reduction in number of plate-

TABLE 2

CONTROL EXPERIMENTS

PLATELET COUNTS (COUNT × 10^3) IN RABBITS AFTER ADMINISTRATION OF VARIOUS SUBSTANCES

Material injected		Saline			Glucose 5%	India Ink	Lecithin 10%	Sphingomyelin	Dog's Spleen†
Date		7/46	12/46	2/47	9/46	9/46	8/45	7/46	12/46
Amount (ml.)		6	10	15	15	*	8	10	Equiv. of 12 Gm.
Rabbit No.		VII	XIII	XXI	XI	X	III	VI	XIV
	hr.								
Reading before injection		625	720	540	575	603	640	670	750
Readings after injection	¼				555	10			
	½		680					636	685
	1	596	695		588	46		625	675
	1½			538					
	2	605					590	645	
	3½				595	196			
	5		705				605		715
	6½	655						660	
	24	685	735	526	562	558	633	645	745
	48			528					
	72		708	533					760

* 2 ml. of India Ink (Higgins) in 15 ml. 5% glucose solution per 6 lb. of rabbit.

† Extracted with acetone.

TABLE 3

PLATELET COUNTS (COUNT × 10^3) IN RABBITS AFTER ADMINISTRATION OF AQUEOUS EXTRACTS PREPARED FROM SPLEENS OF PATIENTS WITH DIFFERENT SPLENIC CONDITIONS

Patient / Date		Ca 12/46	M 1/47		B 3/47		K 4/47	R 4/47	D 4/47	L 4/47	X 12/46	Y 2/47
Diagnosis		Thrombocytopenic purpura							Splenic		Normal	
									np*	hp*	dog	human
Spleen	Weight (Gm.)	—	140		145		180	144	138	300	—	—
	Equivalent injected (Gm.)	15	10	10	10	8	1/10 2/5→ 37.5→	10	10	10	12	10
Rabbit No.		XVI	XVIII	XIX	XXII	XXIII	XXIV	XXV	XXVII	XXVIII	XV	XX
	hr.											
Reading before injection		655	538	695	622	541	595	509	536	588	635	555
Readings after injection	½	642			640						640	
	1	652				575	608			578	620	
	1½		555	689								560
	3				602	563					615	
	24	575	428	301	614	532	→	383	538	553	601	554
	48	435	205 (−61%)	283 (−58%)	481	351 (−35%)	412	347 (−31%)	536	564	629	573
	72	419 (−36%)			393 (−37%)	418	→ 430	539	540		619	546
	96			457		425	367 (−38%)			582		
	120	543			644	547	566					568
	192		571				588					
	240	646		710								

* Second and third injections were given after the 24 and 72 hour samples were taken; np, indicates neutropenia; hp, hematopenia; (1), (2) and (3) first, second and third injections, the second injection having been administered after the 24-hour sample had been taken and the third injection after the 72-hour sample had been taken.

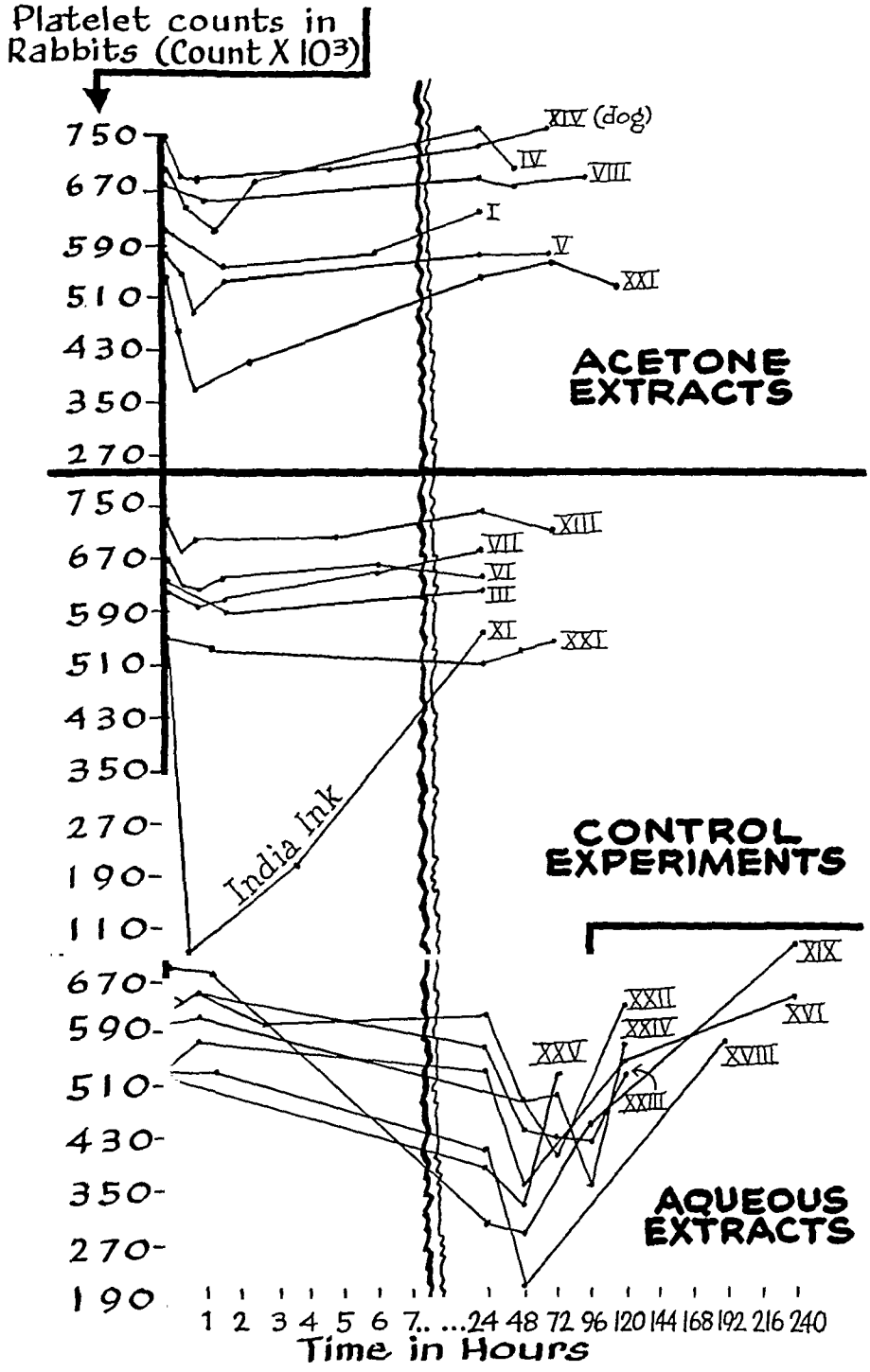


FIG. 1. Effect of splenic extracts and india ink on platelet counts in rabbits

lets within the first fifteen minutes; even in this case, however, recovery started as early as one hour after injection and reached normal levels within 24 hours. Similar findings were reported[17] with the use of colloidal silica suspensions; here, the extent of platelet reduction was apparently proportional to the size of particles employed. In addition Table 2 contains observations with emulsions of lecithin and sphingomyelin and an extract from spleens of normal dogs. The decrease of platelets in these cases was slight and similar to that obtained with the extracts of diseased human spleens. All these findings seem further to substantiate the assumption that the causes of immediate platelet reduction are not specific and should be distinguished from the effects described below.

A change in the extraction technic was believed to be necessary. In accordance with the observations of Torrioli *et al.*,[19, 20] there was a chance that the aqueous extracts of the spleen might yield a more potent platelet-reducing agent than the acetone extracts. The problem was to prepare a concentrated, protein-free splenic extract without exposing the material to higher temperatures. The Na_2SO_4 method of extraction, which has been used successfully in certain hormone preparations, served this purpose satisfactorily.

Table 3 presents our results with nine such extracts. Five of them were made from spleens of patients with essential thrombocytopenic purpura, two from patients with other conditions, and two spleens were from normal controls. From this table, it is obvious that there was no initial drop in the platelet count in any of these animals. The platelet reduction did not begin earlier than 24 hours after the injection and in some animals it was delayed for 48 or 72 hours. The maximum reduction was reached gradually and lasted two to three days. It is significant that the decrease ranged from 31 to 61 per cent. The effect of extracts M and B was reproducible in two animals. Recovery after the depression was usually somewhat faster and in some animals (XXIV, XXV) occurred within 24 hours. Also, in order to determine if the effect could be increased or prolonged, rabbit XXIV received three consecutive injections at 0, 24, and 72 hours. The number of platelets did not fall lower than after a single injection (−38 per cent) and reverted to normal by the end of 120 hours. (Rubegni[15] reported a similar observation.) It was noted, however, that in this animal a marked anemia developed which was later characterized by reticulocytosis (27 per cent). Following the return of the platelet count to normal, the anemia also improved. The splenic extracts from Cases D, L, and Y, and the dog spleen X, had neither an initial nor a delayed effect and served as controls.

Repeated qualitative and quantitative white blood cell studies in all these rabbits, including the one developing anemia, eliminated the possibility of an intercurrent infection.

Figure 1 contains diagrams of data presented in the three tables.

DISCUSSION

Our studies did not substantiate the original claim of Troland and Lee[22] and their followers[2, 5, 9, 11, 12, 14, 15, 23] that the acetone extract of spleens from essential thrombocytopenic purpura patients would exert a specific platelet-reducing effect in rabbits. Our findings, rather, support the conclusions of the group

of investigators[1, 6, 8, 10, 13, 18, 25] who were unable to reproduce Troland and Lee's experiments. It is our belief that the slight, though frequent, initial decrease in platelet count is nonspecific in nature and depends on the physical properties rather than the chemical composition of the injected material. On the other hand, we were able to confirm the findings of Torrioli and Puddu[20] by demonstrating a delayed thrombocytopenia in rabbits following the intravenous injection of protein-free aqueous concentrates from thrombocytopenic spleens. Our improved extraction-technic may account for the fact that splenic extracts proved to be more effective in smaller equivalents than had heretofore been observed. Contrary to Torrioli[20] and to Hobson and Witts,[5] however, we could not obtain such results when using extracts made from normal spleens; we purposely abstained from using excessive doses of these extracts in our experiments. Hence, there can be little doubt that the active principle in the aqueous extract is either specific or more abundantly present in thrombocytopenic spleens. The agent is definitely water-soluble and protein-free which means that it is unlikely that it is a lipid as was formerly believed.

We are unable, as yet, to evaluate the relationship of our active agent to those recently described by Ungar.[24] Using a different technic, Ungar apparently succeeded in demonstrating two principles in the spleen, and assumed that they had hormonal affinities with the pituitary and adrenal cortex. His findings, though highly suggestive in regard to splenic function, require further study for confirmation.

The chief difficulty with splenic extracts is that they do not necessarily reflect the entire splenic activity. They might represent a partial function which was segregated from its internal environment, or they might be artifacts and not even exist *in vivo*. In neither case would it seem permissible to draw final conclusions regarding the pathogenesis of a disease such as thrombocytopenic purpura. The only thing which can be stated safely at present is that the extract does not produce direct excessive platelet destruction in rabbits; otherwise, that process should be most evident soon after injection. The delayed action may perhaps be explained by assuming a temporary depression of platelet formation after which the platelets return to normal.[7] In accordance with this concept, it has been reported that the bone marrow of rabbits does not show any significant change in the number and structure of the megakaryocytes after a single injection of a splenic extract;[12] the transformation into platelets seems to be halted only temporarily. In higher concentrations, of course, the megakaryocytes will be injured directly, as Torrioli and Pusic[21] have demonstrated in tissue culture studies. The transitory nature of the bone marrow injury is also supported by the observation that, after repeated injections of splenic extracts into rabbits, the platelet count began to rise again and marked reticulocytosis ensued. There was also an indication of overproduction of platelets in some animals. The agent apparently acts as a mild toxic irritant (splenotoxin?).

SUMMARY

From spleens of patients with thrombocytopenic purpura, two types of extracts were prepared: (1) acetone extracts which produced only an initial and

transitory reduction of platelets in rabbits and (2) aqueous extracts, prepared by the sodium sulfate concentration method, which yielded a much more potent agent with delayed and more prolonged platelet-reducing effect in the same animal. Extracts from spleens from normal persons or patients with neutropenia gave similar results to those obtained with the acetone extracts but negative results with the aqueous extracts. These findings indicate that there is a specific agent in spleens of patients with thrombocytopenic purpura.

REFERENCES

1. Colmer, M. L., and Mersheimer, W. L.: Relation of splenic extract to etiology of essential thrombopenia. Arch. Surg., **43:** 422–426, 1941.
2. Cronkite, E. P.: Further studies of platelet-reducing substances in splenic extracts. Ann. Int. Med., **20:** 52–62, 1944.
3. Deutsch, A., Eggleton, M. G., and Eggleton, P.: The use of sodium sulphate for the preparation of concentrated protein-free tissue extracts. Biochem. J., **32:** 203–207, 1938.
4. Frank, E.: Die essentielle Thrombopenie. Berl. klin. Wchnschr., **52:** 454–457, 1915.
5. Hobson, F. C. G., and Witts, L. J.: Platelet-reducing extracts of the spleen. Brit. M. J. **1:** 50–51, 1940.
6. Hodge, I. G., and Strong, P. T.: The effect of splenic extracts of patients with thrombocytopenic purpura on the platelet count of rabbits. Bull. Ayer. Clin. Lab., Pennsylvania Hosp., **3:** 267–276, 1939.
7. Limarzi, L. R., and Schleicher, E. M.: Bone marrow in the active phase of essential thrombocytopenic purpura. J.A.M.A., **112:** 879–888, 1939.
8. Major, R. H., and Weber, C. J.: Is there a platelet-reducing substance in the spleen of thrombocytopenic purpura? J. Lab. and Clin. Med., **25:** 10–13, 1939.
9. Moolten, S. E.: Studies on extractable factors in spleen and other organic sources which influence blood platelet count. J. Mt. Sinai Hosp., **12:** 866–873, 1945.
10. Moore, C. V.: Discussion abstract. J. A. M. A., **114:** 12, 1940.
11. Otenasek, F. and Lee, F. C.: Further observations on thrombocytopen. J. Lab. and Clin. Med., **26:** 1266–1273, 1941.
12. Paul, J. T.: The effect of splenic extracts from cases of essential thrombocytopenic purpura on the platelets and hematopoietic organs of rabbits. J. Lab. and Clin. Med., **27:** 754–762, 1942.
13. Pohle, F. J., and Meyer, O. O.: Inability to demonstrate a platelet reducing substance in an acetone extract of the spleen from patients with idiopathic thrombocytopenic purpura. J. Clin. Investigation, **18:** 537–541, 1939.
14. Rose, H., and Boyer, L. B.: Thrombocytopen: A confirmatory report. J. Clin. Investigation, **20:** 81–86, 1941.
15. Rubegni, R.: Sull'esistenza a sul modo d'azione di un fattore trombocitopenico negli estratti di milza e di altri organi. Policlinico (sez. med.), **47:** 1–10, 1940.
16. Scheff, G. J., and Ralph, P. H.: An improved method of counting blood platelets and its evaluation in blood of rabbits. Am. J. Clin. Path., **19:** 1113–1121, 1949.
17. Tait, J., and Elvidge, A. R.: Effect upon platelets and on blood coagulation of injecting foreign particles into the blood stream. J. Physiol., **62:** 129–144, 1926/27.
18. Tocantins, L. M.: No platelet-destroying action in extracts of the spleen and urine of patients with chronic thrombocytopenic purpura. Proc. Soc. Exper. Biol. and Med. **42:** 485–486, 1939.
19. Torrioli, M., and Puddu, V.: Studio sulla biologia dei megacariociti sopravviventi in vitro. Policlinico (sez. med.), **41:** 245–254, 1934.
20. Torrioli, M., and Puddu, V.: Recent studies on pathogenesis of Werlhof's disease. J. A. M. A., **111:** 1455–1456, 1938.
21. Torrioli, M., and Pusic, G.: Azione piastrinopenica elettiva di dosi massive di estratto splenico. Boll. Soc. Ital. Biol. Sper., **9:** 662–670, 1934.
22. Troland, C. E., and Lee, F. C.: A preliminary report on a platelet-reducing substance in the spleen of thrombocytopenic purpura. Bull. Johns Hopkins Hosp., **62:** 85–86, 1938; also, Thrombocytopen. J. A. M. A., **111:** 221–226, 1938.
23. Uihlein, A.: The effect of injection of tissue extracts on the number of blood platelets. J. Lab. and Clin. Med., **28:** 157–162, 1942.
24. Ungar, G.: Études biochimiques et physiologiques sur deux substances actives produites par la rate. J. de physiol. et de path. gén., **39:** 219–225, 1947.
25. Watson, G. M.: The blood platelets and splenic extracts. Brit. M. J., **1:** 704–706, 1941.

REPLACEMENT TRANSFUSION FOLLOWING ADMINISTRATION OF INCOMPATIBLE BLOOD

Report of Case With Successful Outcome

JAMES E. CONLEY, M.D., JOHN ERBES, M.D., F. JACKSON STODDARD, M.D., AND JOHN W. FRYE, M.D.

From the Department of Surgery, Columbia Hospital, and Marquette University School of Medicine, Milwaukee, Wisconsin

The following case is presented as an example of replacement transfusion as an effective and practical method of treating reactions from transfusion of incompatible blood.

REPORT OF CASE

L. T., a 35 year old housewife, para III, was admitted to the hospital on August 27, 1948 because of severe lower abdominal pain and vaginal bleeding of two hours' duration. She was admitted with a diagnosis of possible ectopic pregnancy. On examination she was well developed and nourished and appeared acutely ill. The abdomen was markedly tender in both lower quadrants, especially on the right side where spasm of the rectus muscle was elicited. An emergency operation was performed. It revealed a ruptured Graafian follicle cyst with intraabdominal hemorrhage of 700–800 cc. The patient was given a transfusion of 500 cc. of type AB, Rh-positive blood during operation. The blood pressure fell to 80/50 toward the end of the operation and the pulse rate rose to 120 per minute.

The transfusion was followed by an intravenous infusion of 1000 cc. of 5 per cent dextrose in 0.85 per cent saline. This was completed before recovery from anesthesia. A second transfusion of 500 cc. of type O, Rh-positive blood was given two hours after operation to help replace the blood lost during operation. The blood pressure was 100/70 when this second transfusion was begun. An infusion of 1000 cc. of 5 per cent dextrose in distilled water was given after the transfusion. The blood pressure reading was 106 to 118 mm. systolic and 65 to 70 mm. diastolic.

Since the patient did not void for 10 hours after the operation, she was catheterized and 200 cc. of reddish brown urine was obtained. This was the first obvious evidence of an incompatible transfusion reaction. Urinalysis revealed hemoglobinuria and 2500 mg. of albumin per 100 cc. of urine. The icterus index was 22.3 and the nonprotein nitrogen was 39.1 mg. per 100 ml. blood (Fig. 1). Re-typing of the patient's blood revealed that it was type O instead of type AB, indicating that the first transfusion of 500 cc. given during the operation, contained incompatible blood. The immediate treatment instituted was an infusion of 1000 cc. of 1/5 molar sodium lactate solution followed by 1000 cc. of 10 per cent glucose in distilled water. Sodium bicarbonate was given by mouth to alkalinize the urine.

During the first 16 hours after the operation, the patient received a total of 5000 cc. of fluid intravenously, of which 1000 cc. was blood. The pulse rate ranged from 100 to 120, but the blood pressure returned to the preoperative level. However, less than 400 cc. of urine was obtained per catheter. The urine was reddish brown in appearance and of low specific gravity, 1.008. It was thought that the patient had oliguria resulting from transfusion with incompatible blood. Therefore, a replacement transfusion was performed 16 hours after operation.

The patient was returned to the operating room and oxygen was administered by a closed anesthesia system. An amount of 1 per cent sodium pentothal sufficient to render the patient drowsy was given. A 16-gauge blunt-nosed needle was inserted into the right saph-

* Received for publication, September 7, 1949.

enous vein at the right ankle and transfusion was begun. Another transfusion was started in a vein on the dorsum of the left foot. The right radial artery was exposed and a cannula inserted for exsanguination. Transfusions were then administered as rapidly as the various systems would permit. After 1000 cc. of blood had been administered, bleeding was instituted from the right radial artery. In less than ten minutes 1500 cc. was withdrawn and the exsanguination was stopped temporarily. The administration of blood was continuous, and when a total of 3000 cc. had been given, more bleeding was permitted from the right radial artery until an additional 1000 cc. of blood had been removed. After a total of 5000 cc. of blood had been given, which was about 2000 cc. above the amount withdrawn, considerable difficulty was encountered in obtaining blood from the radial arterial cannula which became clotted frequently. The removal of blood was more difficult than its administration. Heparin as recommended by Weiner and Wexler[4] was not used because of the recent operation.

The patient became somewhat dyspneic and rales were heard over both bases. These were interpreted to indicate pulmonary edema secondary to the sudden increase in blood

TABLE 1

BLOOD STUDIES

DATE	AUG. 27 BEFORE	AUG. 27 AFTER	AUG. 28	AUG. 30	AUG. 31	SEPT. 1	SEPT. 2	SEPT. 4	SEPT. 6	OCT. 6, 1948	APR. 9, 1949
	Replacement Transfusion								Discharged		
Nonprotein nitrogen, mg.	39.1	33.5	36.7	49.0	29.9	32.0	39.1	34.3		31.3	22.4
Icteric index	22.3	20.7	14.3	8.3	9.5	8.5	8.0	10.0		6.8	4.8
Prothrombin, %			25	42		34		41		70	100
Protein, Gm.						6.0				6.8	6.2
Albumin, Gm.						2.0				2.8	3.3
Globulin, Gm.						4.0				4.0	2.9
RBC, millions	3.6		3.9	3.9	4.8	4.5	5.3	5.0			
Hb., Gm.	9.0		10.5	12.0	14.0	12.5	14.0	13.8			

volume (2000 cc.). Consequently, the radial artery was released so that bleeding was obtained directly from the artery rather than via the cannula. By this means bleeding was copious in amount. After the removal of another 2000 cc. of blood, there was immediate improvement in the patient's condition. The administration of blood was continued until 6000 cc. had been given. A total of 12 transfusions or 6000 cc. of type O, Rh-positive blood was administered and a total of 5000 cc. was removed. The blood in all of the 12 transfusions administered had titers of less than 1:200 anti-A and anti-B agglutinins. The entire procedure required less than 90 minutes.

On the first postoperative day (August 28, 1948) the patient's condition was satisfactory. The urine was clear and amber in color. There was no hemoglobinuria or other pigment. In the six hours immediately following the replacement transfusion the urine output was 2200 cc. as determined by catheterization. In the next 24 hours the urine output by catheter was 2100 cc.

On August 29, 1948 the patient took about 700 cc. of fluid by mouth. This was supplemented by intravenous infusion of 2000 cc. of 5 per cent glucose and normal sodium chloride solution. The urine was clear and no longer contained hemoglobin. The urine output was 1225 cc. during the second 24-hour period following surgery.

On the third postoperative day (August 30, 1948), there was slight abdominal distention. This was relieved by a soap-suds enema which resulted in a copious evacuation. The highest temperature recorded was 99.6 F. during the first three days. The pulse rate was 90 per minute and the blood pressure 108/74. The oral intake of fluid exceeded 1500 cc.

Further convalescence was uneventful. The patient was discharged nine days postoperatively. She was well and active in spite of having had a major pelvic operation, the administration of an incompatible blood transfusion of 500 cc. and an exsanguination transfusion which replaced approximately 65 per cent of her circulating blood volume.

DISCUSSION

In our patient we were able to prevent the development of anuria even though oliguria had occurred following the administration of a transfusion of incompatible blood. During the first 16 hours after the emergency pelvic operation was performed, the patient received a total of 5000 cc. of intravenous fluids, and 600 cc. by mouth, without any vomiting. Only 400 cc. of reddish brown urine with a specific gravity of 1.008 was obtained per catheter. A diuresis of 2200 cc. of clear urine with a specific gravity of 1.012 occurred in the first six hours following the exchange transfusion which replaced approximately 65 per cent of the circulating blood volume. Thereafter, the urine remained clear and the daily output exceeded 1200 cc.

Oliguria following intravascular hemolysis usually lasts 8 to 12 days before spontaneous remission occurs in cases with favorable outcome.[1] The sudden relief of oliguria, and the development of diuresis within a few hours after replacement transfusion could scarcely be considered coincidental in the case being reported. In 34 cases of hemoglobinuric nephrosis reported by Burwell, Kinney, and Finch[1] in which the patients recovered, diuresis began from the second to the twelfth day in 32 cases, and it began on the fourteenth and the sixteenth days in two cases. In two cases reported by Kugel[2] appreciable amounts of urine were first passed on the ninth and tenth days.

The duration of renal insufficiency in the case reported was too brief to cause an appreciable change in the blood chemistry. The nonprotein nitrogen which was within normal limits before replacement transfusion remained unchanged. The icterus index, which was 22.3 before exchange transfusion, was 14.3 on the first day after replacement therapy and gradually dropped to normal (Table 1).

The first urine obtained by catheterization ten hours after the transfusion of incompatible blood was of reddish brown color with low specific gravity. Shortly thereafter the urine became somewhat clearer but contained large masses of suspended black pigment and casts. There was 2500 mg. of albumin per 100 cc. urine and a trace of hemosiderin. Within a few hours after replacement transfusion, the urine was amber with no pigmented casts and contained only 10 mg. of albumin per 100 cc. In the favorable cases hemoglobinemia and its attendant hemoglobinuria usually disappear within 24 to 36 hours. However, proteinuria continues, even after the hemoglobinuria subsides.[3] It amounts at first to 500 to 1000 mg. per 100 cc. of urine and gradually decreases to 50 to 100 mg. per 100 cc. within four to five days, according to Muirhead, Haley, Haberman, and Hill.[3] In the reported case albuminuria had disappeared within six hours after com-

pletion of the replacement transfusion. According to Muirhead, Haley, Haberman and Hill, when the urine specific gravity is depressed (1.005–1.010), it remains low after recovery. In the present case the specific gravity was noted at 1.008 during the oliguria and rose to 1.012 during the phase of diuresis after replacement transfusion. It remained within normal range thereafter.

An interesting coincidental observation during convalescence was a persistently low prothrombin time which appeared to be refractory to Vitamin K administration. Liver function studies were not performed but intrahepatic damage may follow hemolytic transfusion reactions. A cholecystogram performed two months after discharge from the hospital revealed a normal gallbladder with no apparent calculi. An albumin and globulin determination done on the sixth posttransfusion day revealed a reversal of the A/G ratio (Table 1). The test repeated one month later showed normal blood protein value but a persistent reversal of the A/G ratio.

SUMMARY

Replacement transfusion can be performed in adults without appreciable risk. This method of removing the nephrotoxic products of intravascular hemolysis was employed in a patient with favorable outcome. Relief of oliguria, proteinuria, and hemoglobinuria occurred immediately following replacement transfusion therapy. Further experience and observation are needed to ascertain the value of replacement transfusion in the emergency treatment of reactions following transfusion of incompatible blood.

REFERENCES

1. Burwell, E. L., Kinney, T. D., and Finch, C. A.: Renal damage following intravascular hemolysis. New England J. Med., **237**: 657–665, 1947.
2. Kugel, V. H.: Management of acute toxic nephrosis. Am. J. Med., **3**: 188–205, 1947.
3. Muirhead, E. E., Haley, A. E., Haberman, S., and Hill, J. M.: Acute renal insufficiency due to incompatible transfusion and other causes, with particular emphasis on management. Blood, **2**: 101–138, 1948.
4. Wiener, A. S., and Wexler, I. B.: The use of heparin when performing exchange blood transfusions in newborn infants. J. Lab. and Clin. Med., **31**: 1016–1019, 1946.

BEHAVIOR OF VIRUS OF POLIOMYELITIS IN A CULTURE OF *ENDAMOEBA HISTOLYTICA**

VIOLA MAE YOUNG, M.S., OSCAR FELSENFELD, M.D., AND CHESTER L. BYRD, JR., M. T. (ASCP)

From the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois

Several authors have attempted to propagate the virus of poliomyelitis on various intestinal bacteria, protozoa and free-living microorganisms. The results of recent experiments in this field have been published by Brutsaert *et al.*[1] and Toomey *et al.*[3] who also surveyed the literature concerning this subject. The results of these investigations were not encouraging with the exception of the experiments involving *Trichomonas hominis*. A mouse-adapted strain of human poliomyelitis virus survived three serial passages with this protozoon.[1] *Amoeba proteus* also carried the Lansing virus mouse strain for a brief period when massively infected.[3]

Our primary interest centered around the exploration of the behavior of the Lansing virus in young artificial cultures of *Endamoeba histolytica* which has not been studied as yet by quantitative means.

MATERIALS AND MEDIA

The virus was received through the courtesy of Dr. S. O. Levinson from the Michael Reese Research Foundation of Chicago. This strain showed a remarkable stability and was also easily adapted to animals other than mice and monkeys.[4] The *E. histolytica* culture was received from Dr. W. Balamuth of Northwestern University. This strain proved to have a constant growth curve and was grown by us in the presence of only two bacteria, *i.e.*, a rough *Escherichia coli* and a *Bacillus subtilis*-like gram positive rod, differing from standard *B. subtilis* strains by its homogeneous growth in liquid media. In order to achieve a sufficiently luxuriant culture of the amebas, it was decided not to use the bacteria-free technic but to grow the amebas in the presence of the above two microbes on egg-Locke slants covered with serum-Locke solution,[2] sterilize the growth with antibiotics, and extract the virus with ether.

Five per cent suspensions of mouse brains from animals paralyzed from one to five days after inoculation, were prepared in sterile physiologic saline. The amebas were grown for 24 hours on egg-Locke slants of equal length covered with 4.5 ml. of serum-Locke solution. The mixtures were incubated and, after varying periods, the amebas and the supernatant fluid separated. This was carried out by shaking the culture tubes, adding 500 micrograms of streptomycin and 500 units of penicillin per ml., letting stand for one hour at room temperature, and centrifuging for 15 minutes at 1000 r.p.m.

* Supported by a grant from the Dr. Leonard H. and Louis Weissman Medical Research Foundation.

Received for publication, September 30, 1949.

Two ml. of anesthetic ether was added to both the sediment and the supernatant. After shaking, the ether layers were pipetted off and the ether evaporated *in vacuo* at room temperature. Remaining sediments were suspended in 2 ml. saline, and 0.03 ml. of the suspensions inoculated intracerebrally into groups of 18 Plymouth-strain white mice. The experiment was repeated twice with groups of 24 mice each, at two month intervals.

Control experiments were set up with cultures containing the following: (1) virus and bacteria but no amebas, and (2) virus but no bacteria or amebas. The same medium, the same separation technic and the same number of animals were employed in each instance.

TABLE 1

MEAN SURVIVAL TIME OF MICE INOCULATED WITH MIXTURES OF LANSING VIRUS, *E. histolytica* AND TWO BACTERIA

MIXTURE	10 MIN.	6 HR.	24 HR.	48 HR.
	Mean Survival Time (in Days)			
V* + A.C.† with bacteria, sediment	6.3 ± 0.8	3.8 ± 0.5	5.4 ± 0.8	6.4 ± 0.8
V + A.C. with bacteria, supernatant	6.2 ± 0.3	8.6 ± 1.1	9.8 ± 1.0	10.2 ± 1.2
V + bacteria from A.C., no amebas, sediment	5.8 ± 0.3	5.2 ± 0.8	6.8 ± 0.9	10.6 ± 1.1
V + bacteria from A.C., no amebas, supernatant	6.1 ± 0.4	7.2 ± 0.9	12.1 ± 1.2	10.9 ± 1.1
V + sterile culture fluid, no amebas or microbes, sediment	6.0 ± 0.6	5.6 ± 0.5	12.1 ± 1.1	11.6 ± 1.4
V + sterile culture fluid, no amebas or microbes, supernatant	5.8 ± 0.6	5.8 ± 0.5	11.8 ± 1.2	12.0 ± 1.6
Mean amebic count, thousands	47.1 ± 8.3	50.8 ± 6.3	68 ± 9.2	72.3 ± 9.5
Mean bacterial count, billions	7 + 0.3	9 + 0.5	12 + 1.7	15 + 1.3

V* = virus.
A.C.† = amebic culture.

RESULTS

Table 1 shows the results of these experiments in a total of 1584 mice. Only animals showing typical paralysis were considered for the calculation of the mean survival time. About 5 per cent of the animals receiving injections of extracts of cultures containing *E. histolytica* died within 24 hours after inoculation, probably due to a toxic action of the ether extract of this protozoon. An inquiry into the nature of this toxic substance or substances is under way. Two animals were lost, of those injected with the mixture of bacteria and virus without amebas, and 3 mice died of those inoculated with virus without bacteria and amebas, due to brain injury following inoculation.

There was no significant difference in the mean survival time of animals inocu-

lated with virus-ameba-microbe mixture, virus-ameba mixture, virus-microbe mixture, and virus alone when these mixtures were incubated for only 10 minutes. After 6 hours' incubation, however, the sediment of the amebic cultures containing virus killed mice within a significantly shorter period of time than the supernatant of the same cultures which had been freed of amebas by gentle centrifugation (sufficient to separate the amebas but not strong enough to cause sedimentation of the virus unattached to the protozoons, 1000 r.p.m.). Presuming that the length of the survival time of the mice is related to the concentration of the virus, the prolonged survival time of animals inoculated with the supernatant fluid from virus-ameba-microbe mixtures, as compared with the life span of animals inoculated with virus alone, suggests that at this point the virus was more concentrated, or absorbed, within or upon the surface of the amebas. The comparison of the effect of virus-microbe mixtures shows a similar phenomenon, but to a lesser extent.

After 24 and 48 hours' incubation, all mixtures became less virulent. While after 24 hours the sediments of both virus-ameba-microbe and virus-microbe mixtures showed an increased ability to kill mice, after 48 hours only the sediments of the virus-ameba-microbe mixtures showed a greater ability. The supernatants of the above cultures did not follow the same pattern. It must be noted that in spite of a certain decrease in the concentration of the virus upon exposure to incubator temperature, such a decrease was slowed in the presence of amebas and bacteria. This is particularly true in the case of amebas, which appeared to retain a higher concentration of the virus for the full experimental period. This phenomenon is probably due to protection by absorption of the virus by the organisms.

In order to ascertain whether the virus multiplied in the protozoa, cultures of amebas and of the "nutrient organisms" with Lansing virus, as well as Lansing virus alone, were prepared in the manner described above. The sediments and supernatants, however, were not separated but the entire liquid part of the medium was treated with antibiotics, extracted with ether and suspended in saline, as in the main experiment. Serial dilutions of each suspension were injected in groups of 6 mice each and the 50 per cent lethal doses calculated. It was found that the $L.D._{50}$ titers of the ameba-bacteria-virus and the bacteria-virus mixtures, and of virus alone, were 3.8, 4.3 and 3.9, respectively. Thus the Lansing type virus of poliomyelitis does not seem to multiply in the cultures of these amebas and bacteria, and the difference between the concentration of the virus in the sediments containing amebas and/or bacteria has to be ascribed to absorption or adsorption of the virus by these microorganisms.

SUMMARY

Experiments were carried out with a strain of *Endamoeba histolytica* cultured in the presence of a rough *Escherichia coli* strain and a *Bacillus subtilis*-like organism, and the Lansing strain of poliomyelitis virus. The amebas and the bacteria seem to absorb or adsorb the virus; the first for a longer, the latter for a shorter period of time.

Acknowledgment. The authors are indebted to Dr. Einar Leifson, Chairman of the Department of Bacteriology of the Loyola University School of Medicine for his valuable advice relating to certain phases of this work.

REFERENCES

1. Brutsaert, P., Jungeblut, C. W. and Knox, A.: Attempts to propagate murine poliomyelitis virus on various intestinal bacteria and protozoa. Proc. Soc. Exper. Biol. and Med., **61**: 265–269, 1948.
2. Felsenfeld, O., and Young, V. M.: A quantitative method for the determination of the amebacidal activity of drugs in vitro. Tr. Am. Micro. Soc., **44**: 286–289, 1945.
3. Toomey, J. A., Takacs, W. S., and Schaeffer, M.: Attempts to infect Amoeba proteus with poliomyelitis virus. Am. J. Dis. Child., **75**: 11–18, 1948.
4. To be published.

CONGENITAL PULMONARY ASPIRATION-TUBERCULOSIS

Report of a Case*

A. J. HERTZOG, M.D., SIMS CHAPMAN, M.D., and JOHN HERRING, M.D.

From the Departments of Pathology, Pediatrics, and Obstetrics of Touro Infirmary, New Orleans, Louisiana

In tuberculous pregnant women tubercle bacilli may involve the endometrium and produce lesions in the placenta. The rarity of such infection is probably explained by the fact that women suffering from genital tuberculosis are usually sterile as a result of the tuberculous salpingitis.

Tuberculosis of the fetus occasionally occurs and represents true congenital tuberculosis. The bacilli usually enter the fetus through the umbilical vein and the primary focus of tuberculosis develops in the liver. In 1935 Beitzke[1] analyzed all published cases and classified them according to the site of the primary complex. He found 61 cases of proven genital tuberculosis in the mothers, with anatomic lesions, and 40 in which tubercle bacilli were demonstrated in the fetus without histologic changes. He accepted cases of congenital tuberculosis in the infant only if the tuberculous nature of the lesions in the infant was proved. He considered that (1) a primary tuberculous complex in the fetal liver is proof of its congenital nature since it can only have arisen from tubercle bacilli in the blood of the umbilical vein; and (2) if there is no primary complex in the liver, the infection is congenital only if (a) the tuberculous changes are found in the fetus *in utero*, at birth, or a few days after birth; (b) in a child who lives longer than a few days if extrauterine infection can be excluded with certainty, the child being immediately separated from the mother and kept in an environment free from the tubercle bacilli. In 1946 Hughesdon[3] concluded that in the last seventy years there were about 115 cases of proved congenital tuberculosis described in the world's literature. Cases of congenital tuberculosis in which the infection is limited entirely to the lungs, as seen in an aspiration type of infection, are extremely rare. In a review of the literature Reichle and Wheelock,[4] in 1939, found only 7 unequivocal cases of tuberculosis of the aspiration type. They reported death of a nineteen-day-old infant, from widespread pulmonary tuberculosis of the aspiration type, the infection being limited to the lungs and bronchial lymph nodes. They were careful to exclude any possibility of extrauterine acquired tuberculosis or a miliary spread to the lung from lesions of the liver. The mother had tuberculosis, and endometrial tuberculosis was proved by postpartum curettage. To this small number of well-established cases of congenital aspiration tuberculosis, we wish to add another.

Report of Case

A male baby was born five weeks prematurely, on January 9, 1949, in Touro Infirmary on the service of Drs. E. L. King, John Herring and Isadore Dyer. It

* Received for publication, July 13, 1949.

was the mother's first pregnancy; her health had been good except for unexplained fever at the beginning and end of pregnancy. The baby weighed 5 lb., 14 oz. at birth, and otherwise appeared normal. The placenta showed no gross pathologic change. The baby was placed in the "premature" nursery and allowed to leave the hospital on January 20, 1949. During its stay in the hospital, the baby gained weight and, on the day of discharge, was circumcized. At home it suffered from gastro-intestinal disturbances and progressive dyspnea, and did not gain weight; on January 26, it developed a rectal temperature of 101 degrees and was returned to Touro on January 27, 1949 on the services of Dr. Sims Chapman because of fever and respiratory difficulty. Physical examination on admission showed a premature weak infant with cyanosis. Temperature was 100.4 F. Fine crepitant rales were heard over the lungs. The remaining physical examination was not significant. Aspiration of the bronchial tree showed no obstructive lesions. X-ray films of the chest showed extensive miliary dissemination with minute areas of infiltration throughout both lung fields, more prominent in the right lower lobe. The clinical impression was miliary tuberculosis and the infant was placed on streptomycin therapy. The temperature reached 104 F., and the dyspnea increased. The infant expired on January 31, 1949 at the age of 22 days.

Postmortem examination. The body was that of a well-developed, slightly premature infant weighing 2700 Gm. The essential findings were confined to the lungs. Both lungs were studded in all lobes with multiple small grayish white nodules, varying in size from 1 to 3 mm. On cut surface these small nodules were diffusely scattered throughout the parenchyma of both lungs (Fig. 1). There were no enlarged lymph nodes. The remaining organs were not remarkable and there was no evidence of any similar involvement of the liver, spleen or brain. Microscopically, the small pulmonary nodules were composed of deep blue necrotic hyaline material with no tubercle formation (Fig. 3). With the Ziehl-Neelsen stain, most of the hyaline material contained myriads of acid-fast bacilli. The lesions appeared to be largely intra-alveolar, as one would expect in an aspiration infection. Direct smears, cultures and guinea pig inoculations from the pulmonary tissue were positive for *Mycobacterium tuberculosis*. Examination, histologically, of the remaining organs showed no evidence of miliary spread of the infection.

The anatomic diagnosis was aspiration congenital pulmonary tuberculosis.

Postpartum maternal history. Postpartum cultures from the cervix were positive for *M. tuberculosis*. X-ray studies of the chest were negative. A postpartum curettage showed the endometrial stroma to contain epitheloid tubercles (Fig. 2). A biopsy of the cervix also showed tuberculosis. A guinea pig inoculated with endometrial curettings developed tuberculosis.

DISCUSSION

Aspiration of infected amniotic fluid in this infant may have occurred during parturition or at an earlier period. Davis and Potter[2] emphasize that intrauterine respiration is a common occurrence. In support of this opinion is the

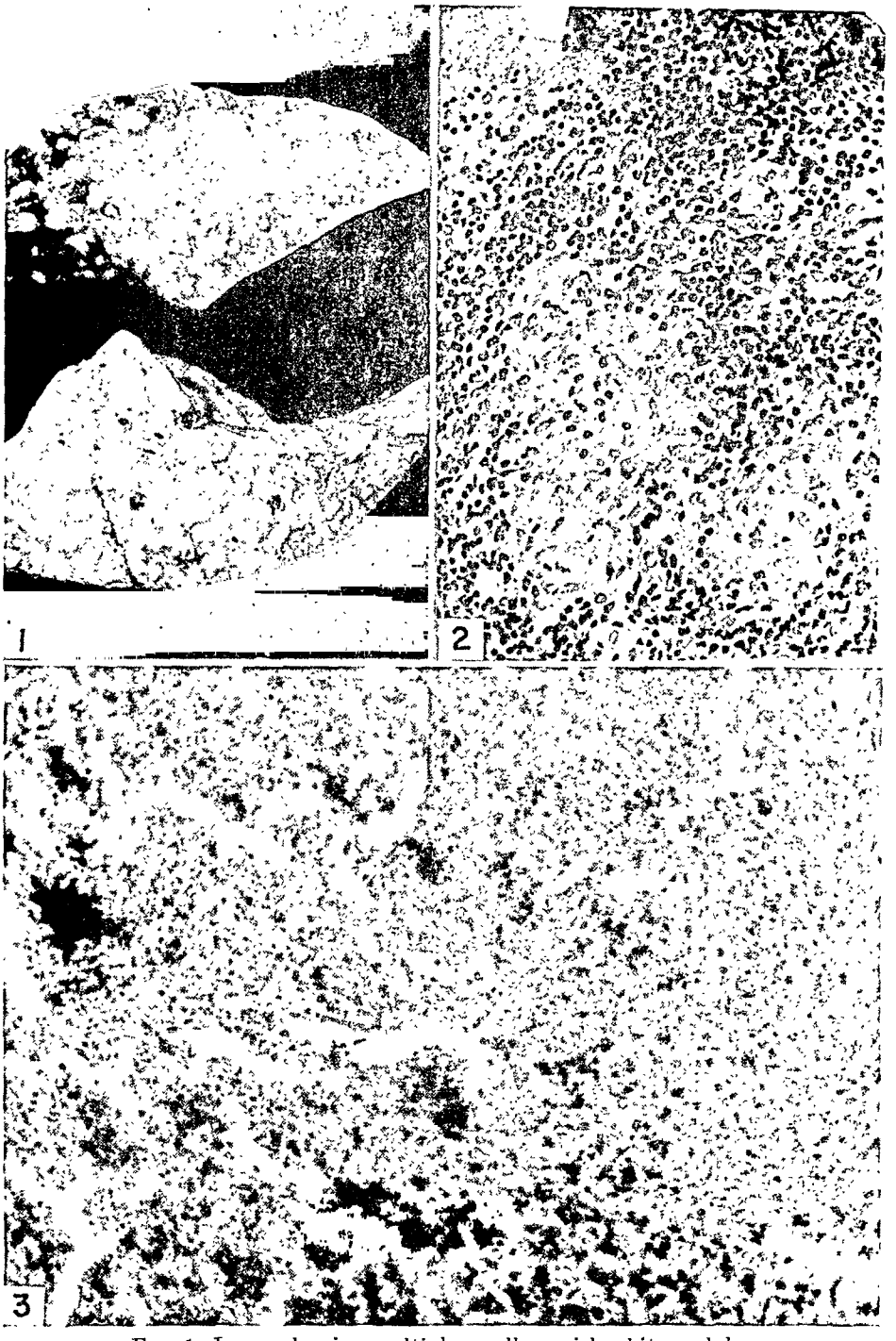


FIG. 1. Lungs showing multiple small grayish white nodules
FIG. 2. Photomicrograph showing tubercles in postpartum endometrial curettings
FIG. 3. Photomicrograph showing structure of pulmonary nodules. The necrotic areas contained large numbers of acid-fast bacilli.

frequent finding of meconium and cornified epithelial cells from the epidermis in the lungs of newborn infants.

The lesions in the lungs with the lack of typical tubercle formation were to be expected when one considers that from animal experiments it has been observed that the tubercle is not the primary reaction of the body to the tubercle bacillus but rather a reaction acquired during the course of the disease. The lesions in our case were quite similar to those described by Reichle and Wheelock.[4] The lesions represent an exudative necrotizing process without the proliferative changes seen in the typical epitheloid tubercle. Similar lesions are seen in guinea pigs inoculated with virulent tubercle bacilli. There was no evidence in this case of a primary complex as seen in postnatal infections. It would appear that the baby received a massive inoculation by aspirating a large amount of heavily infected amniotic fluid. The lack of tuberculosis of any organ, except the lungs, excludes a hematogenous spread. All postnatal contacts in the nursery and at home were checked and found negative for tuberculosis. The mother had no demonstrable tuberculous infection other than uterine tuberculosis.

SUMMARY

An instance is reported of congenital aspiration type of pulmonary tuberculosis in a 22-day-old infant. The mother had proven uterine tuberculosis. Such cases are rare probably because of the frequent development of sterility in women with genital tuberculosis.

REFERENCES

1. Beitzke, H.: Über die angeborene tuberkulöse Infektion. Ergebn. d. ges. Tuberk.-Forsch., **7:** 1–30, 1935.
2. Davis, M. E., and Potter, E. L.: Intrauterine respirations of the human fetus. J. A. M. A. **131:** 1194–1201, 1946.
3. Hughesdon, M. R.: Congenital tuberculosis. Arch. Dis. Childhood, **21:** 121–138, 1946.
4. Reichle, H. S., and Wheelock, M. C.: Aspiration type of congenital tuberculosis. Arch. Path., **28:** 799–807, 1939.

FULMINATING SEPTICEMIA CAUSED BY *MIMA POLYMORPHA*
REPORT OF CASE*

JACOB FAUST, M.D.† AND MARION HOOD, PH.D.

From the Department of Pathology, Charity Hospital, New Orleans, Louisiana

REPORT OF CASE

Clinical Data

A 52 year old, obese, white female was admitted to Charity Hospital on the morning of February 18, 1949. Following a few days of mild infection, apparently of the upper respiratory tract, she was awakened at 4 A.M. with chills and fever, which were followed throughout the day by chest pains, cough productive of mucopurulent sputum, general malaise, nausea, vomiting and dyspnea. Approximately 24 hours later she entered the hospital. Her past history revealed that she had had a hysterectomy in 1943. At that time her blood pressure was 154/92, and the electrocardiogram showed a left bundle branch block. In the interim she had noted mild dyspnea and swelling of the ankles but had received no medication. On physical examination she was markedly dyspneic and dehydrated. The temperature was 102.8 F., pulse rate 95, respiratory rate 45, and blood pressure 76/60. There was mild pharyngeal inflammation. Fine crepitant inspiratory rales were heard posteriorly over the lower half of the left lung. An x-ray film of the lungs showed haziness in both bases. Small hemorrhagic spots were present on the arms and anterior surface of the chest. The laboratory findings were as follows: Urine normal; leukocyte count 20,000 with 78 per cent neutrophils, 16 per cent neutrophilic myelocytes and 6 per cent lymphocytes; carbon dioxide combining power 38 volumes per cent; blood urea nitrogen 15.7 mg. and chloride 526 mg. per 100 ml. The electrocardiogram showed the same pattern as in 1943.

Pneumonia and septicemia were considered as tentative diagnoses. The patient was given sulfadiazine. The systolic level fluctuated between 40 and 60 mm. of mercury even though 500 cc. of plasma was given throughout the night. Anuria developed, apparently as a consequence of the shock. The patient remained alert and rational although she was severely dyspneic. She was then given penicillin every three hours instead of sulfadiazine. Generalized petechial hemorrhages were noted. It was believed that the clinical picture was that of the Waterhouse-Friederichsen's syndrome and she was, therefore, given 15 mg. of desoxycorticosterone, two doses of 5 cc. each of adrenocortical extract and approximately 1000 cc. of whole blood. After six hours of treatment, the blood pressure was 112/80, and the laboratory findings were as follows: 21,000 leukocytes with 79 per cent neutrophils, 14 per cent neutrophilic myelocytes and 7 per cent lymphocytes; the carbon dioxide combining power 38 volumes per cent; blood urea nitrogen 21 mg., and chloride 476 mg. per 100 ml.

The vomiting became more severe and some of the vomitus was aspirated. She developed delirium and progressive abdominal distention. The blood pressure fell to "shock level", the dyspnea became more pronounced with a respiratory rate of 50 per minute, and she developed cyanosis. No fluid was obtained on thoracentesis. The patient died 18 hours after admission to the hospital.

Pathologic Findings

Necropsy was performed three hours after death. The subject measured 5′ 1″ in length and weighed 250 pounds. Multiple petechial hemorrhages were present in the conjunctivae and over the abdomen, legs and back. There was 100 cc. of bloody fluid in the right pleural cavity. The right lung weighed 500 grams, the left lung 400 grams; congestion was noted in

* Received for publication, September 19, 1949.

† Present address, 3305 Carol Drive, Baton Rouge, Louisiana.

the lower lobes. Vomitus was present in both main stem bronchi. The heart showed hypertrophy of the left ventricle and weighed 360 grams. The peritoneal cavity contained 1000 cc. of blood. The liver weighed 2900 grams and showed marked fatty change. The spleen weighed 160 grams, was soft and septic. The adrenals were not hemorrhagic. Microscopic examination showed pulmonary edema, exudate over the surface of the spleen, fatty metamorphosis of the liver, perivascular infiltration and some fresh blood between the cord cells in the adrenals, and arteriolosclerosis.

Bacteriologic Examinations

A blood culture was made soon after the patient was admitted to the hospital. Gram-stained smears made after 12 hours' incubation showed irregularly staining cocci. Subcultures of the organism made on dextrose agar gave luxuriant growth both at room and incubator temperatures. Smears from the agar cultures revealed pleomorphic irregularly-staining bacilli and cocci which ranged in length from 0.8 to 6.0 microns. As the clinical picture suggested Waterhouse-Friederichsen's syndrome, cultures for Neisseria were made on enriched chocolate agar and incubated under partial carbon dioxide tension. After 18 hours, colonies typical of Neisseria were seen which gave a positive oxidase test. Smears made from the oxidase-positive colonies showed organisms morphologically compatible with a Neisseria. Subcultures from these colonies yielded the same abundant growth on dextrose agar at 37 C. and room temperature. Gram-stained smears from the agar again showed irregularly staining pleomorphic bacilli.

DISCUSSION

The organism isolated was plainly not a Neisseria in spite of the positive oxidase reaction and the characteristic appearance of the colonies on the enriched culture medium. The abundant growth of the plain agar subculture both at room and incubator temperatures ruled out the possibility of its being *N. meningitidis*. The predominance of irregularly-staining bacilli shown in the Gram-stained smears from the agar cultures ruled out the possibility that the organism was *N. catarrhalis*. These characteristics did suggest an organism of the tribe. Mimeae. In 1939 De Bord[3] proposed the classification of a new tribe, Mimeae, for an organism which he had isolated. It had appeared to be similar to *Neisseria gonorrhoeae* at first but developed pleomorphic irregularly staining bacilli when cultured on plain agar. Later De Bord[4] reported the isolation of Mima organisms from 90 patients who had either conjunctivitis or vaginitis. If only customary procedures had been carried out, Neisseria would have been reported from all the cases. Deacon[2] reported finding Mima in 19 patients, three of whom had shown resistance to therapy for gonorrhea. A case of Mima meningitis which responded favorably to sulfonamide therapy was reported by De Bord in 1948.[5] Bergey[1] lists this organism under appendix Family Parvobacteriaceae. One of the three species is designated *Mima polymorpha* var. *oxidans*.

A subculture of the organism isolated from the present case was sent to Dr. W. H. Ewing at the Communicable Disease Center of the U. S. Public Health Service for serologic typing. The organisms were agglutinated by antiserums prepared from members of the tribe, Mimeae. Further serologic identification

was not possible. Because our organism was inactive in carbohydrates and gave a positive oxidase test, we classified it as *Mima polymorpha* var. *oxidans*.

SUMMARY

A case is presented of fulminating fatal septicemia caused by an organism of the tribe Mimeae. Since the organism was inactive in carbohydrates and gave a positive oxidase test, it was classified according to Bergey, as *Mima polymorpha* var. *oxidans*.

REFERENCES

1. Bergey's Manual of Determinative Bacteriology. Ed. 6. Baltimore: Williams and Wilkins Co., 1948, p. 595.
2. Deacon, W. E.: A note on the Tribe Mimeae (De Bord). Bact., **49**: 511–512, 1945.
3. De Bord, G. G.: Organisms invalidating the diagnosis of Gonorrhea by the smear method. J. Bact., **38**: 119–120, 1939.
4. De Bord, G. G.: Species of the Tribes Mimeae, Neisserieae and Streptococceae which confuse the diagnosis of gonorrhea by smears. J. Lab. and Clin. Med., **28**: 710–714, 1942–1943.
5. De Bord, G. G.: Mima polymorpha in meningitis. J. Bact., **55**: 764–765, 1948.

CLINICOPATHOLOGIC CONFERENCE*

HANS POPPER, M.D., AND ITALO F. VOLINI, M.D.

From the Departments of Pathology and Medicine and the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago

CLINICAL DATA

History. A 23 year old Puerto Rican woman was admitted with complaints of shortness of breath for three years and recent ankle edema, nausea, vomiting and hemoptysis. Except for dyspnea and an episode of grippe during the past three years, the patient had enjoyed good health. Two weeks before admission, while en route by plane from Puerto Rico to Chicago, she experienced dizziness, epistaxis and severe pain in the chest. She expectorated some blood, became feverish and markedly dyspneic, and the edema increased. Recently, the patient has been unable to retain food and has vomited immediately after ingestion of food.

Physical examination. She appeared acutely ill and slightly icteric. The temperature was 99.4 F., the pulse rate 90, respiratory rate 20 and blood pressure 102/78. Her pupils were round and equal and reacted to light and accommodation; the sclerae were icteric. There were subcutaneous hemorrhages under the left breast. No significant changes were noted on examination of the lungs. The heart borders were normal; a rasping first sound with a systolic murmur and a suggestive diastolic murmur were heard over the pulmonic area and over the apex, and the pulmonic second sound was louder than the aortic second sound. The liver was palpated one fingerbreadth below the costal arch. Generalized abdominal tenderness was present on deep palpation but the spleen and kidneys could not be felt. Pelvic examination revealed bilateral swelling of the labia majora and some bright red blood in the vagina. There were tender external hemorrhoids. There were no abnormal neurologic findings. Both lower extremities were moderately edematous.

Laboratory findings. The urine contained 3 plus albumen. The hemoglobin concentration was 52 per cent, the red cell count 3,140,000, the leukocyte count 7050, with 88 per cent neutrophils, 2 per cent basophils, 4 per cent lymphocytes and 6 per cent monocytes. Two blood Kahn tests were negative while a third was doubtful. Cephalin-cholesterol flocculation was 4 plus, and thymol turbidity 12.8 units. Nonprotein nitrogen was 36 mg. per 100 ml., total protein 6.1 Gm., and the icteric index 32. Repeated urine examinations revealed no bilirubin but 4 plus urobilinogen. Ten days after admission, the nonprotein nitrogen was 28 mg. per 100 ml., the alkaline phosphatase 17 Bodansky units and the icteric index 51. On x-ray examination, increase in peribronchial markings and engorgement of the hilar markings without pulsation were noted. The transverse cardiac diameter was increased and the right border was markedly rounded. The aortic knob could not be made out but a large bulge in the region of the pulmonary artery and conus was noted. The left ventricular border was only moderately

* Received for publication, March 24, 1949.

enlarged, but in the right oblique view, the right ventricle almost obliterated the retrosternal space. No displacement of the esophagus was seen following a swallow of barium. The electrocardiogram revealed right axis deviation.

Course. The patient was treated with vitamin K, iron, digitalis and mercupurin. One night she experienced a sharp precordial pain, had hemoptysis and became dyspneic. Dulness was elicited on percussion over both lung bases but no rales were heard, and ascites became apparent. The liver was palpated 2 cm. below the costal margin. There was temporary improvement during the following days, but later the edema and jaundice increased in severity. A sore throat developed and the patient expired suddenly, 16 days after admission.

CLINICAL DISCUSSION

Dr. Italo F. Volini. "Apparently, we are dealing with 2 conditions, one of cardiac and the other of hepatic nature. There is a history of three years of shortness of breath with a past history of grippe which might conceivably be interpreted as rheumatic fever. She developed symptoms while flying in an airplane and these may have been due to anoxia related to diminution in atmospheric pressure. I am referring to the expectoration of blood and the edema. With this, the electrocardiographic evidence of right heart strain and the findings on physical and fluoroscopic examination, we would appear to have some type of pulmonary hypertension, the etiology of which must be worked out. There is some suggestion that the patient had mitral stenosis, especially since I do not find evidence of bronchopulmonary disease, pulmonary irritation or obliterating endarteritis. Patent ductus arteriosus, which would fit in with some of the findings, is excluded because of the absence of a characteristic murmur. However, despite enlargement of the right ventricle and the bulge in the region of the pulmonary conus, there is no evidence of enlargement of the left atrium on swallowing barium and no hilar pulsation. These findings militate against a diagnosis of mitral stenosis as the cause of the pulmonary hypertension. An associated defect in the interatrial septum, the congenital Lutembacher's syndrome, could explain the present situation better than a simple mitral stenosis on a rheumatic basis. The episodes of hemoptysis might be explained on the basis of pulmonary hypertension or as result of pulmonary embolism. I am inclined to assume the former.

"Should we correlate the liver condition with the pulmonary hypertension or assume the presence of a separate disease? One could consider it a hepatitis of virus etiology although the alkaline phosphatase is very high. Since this recent immigrant most probably received injections for immunization, the possibility of homologous serum jaundice should be considered. The patient also had a hemorrhagic tendency which was not explained by the available hematologic examinations. I am, therefore, assuming the presence of pulmonary hypertension, the etiology of which is not fully explained, and an associated primary parenchymal disease of the liver."

Dr. Edmund F. Foley. "The possibility of altitude sickness in association with the presenting symptoms could suggest that this girl had an aortic aneurysm which ruptured. However, the roentgenologic examination rules that out. I would

agree that the main problem is the association of hepatitis with pulmonary hypertension. Bacterial endocarditis should at least be considered. The episodes which complicated the original picture could also be explained by pulmonary embolism. The minimal liver damage present at the time of admission to the hospital was possibly aggravated by such an embolism."

Dr. Frederick Steigmann. "In liver disease, we have been stressing the question whether the patient had been outside the United States. Some of the unusual conditions found in Panama occur in soldiers stationed in Puerto Rico. There might be some connection between this patient being a Puerto Rican and the symptoms she developed. This may indeed be an exotic infection which involves the lungs and the liver."

A resident. "This patient came from Puerto Rico as a housemaid. Therefore, she probably was immunized."

An intern. "The bleeding from the vagina was due to menstruation which she did not think was unduly prolonged."

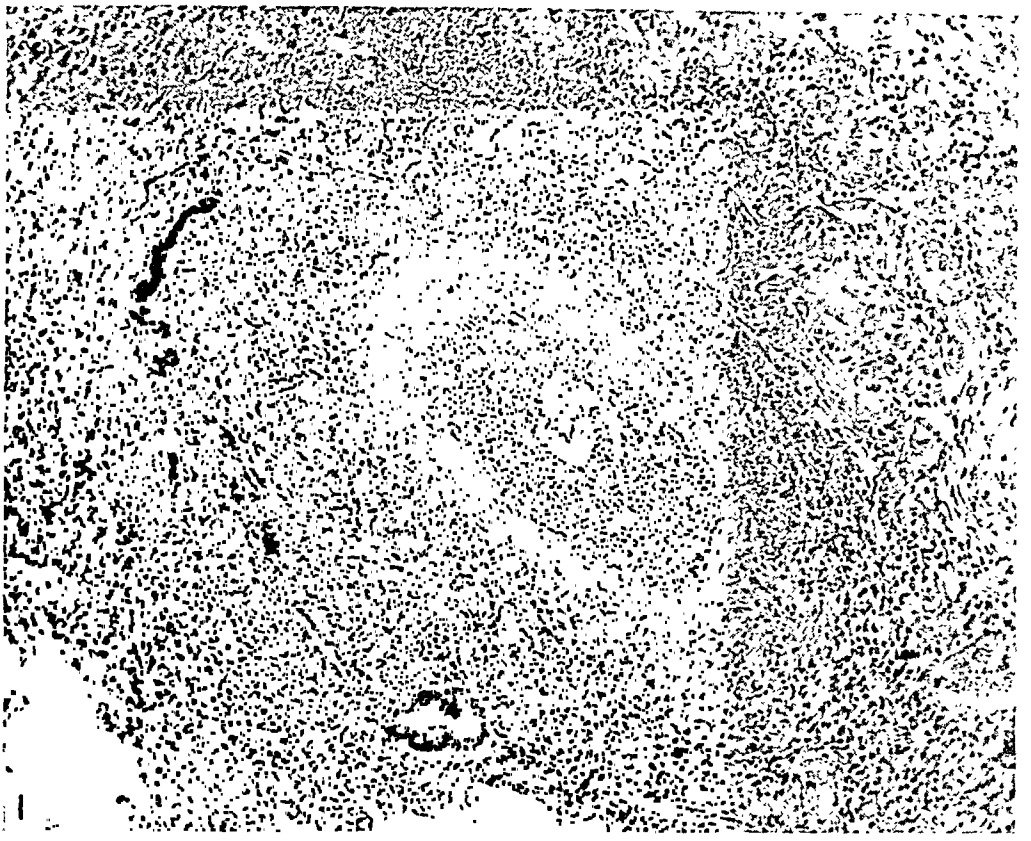
PATHOLOGIC FINDINGS

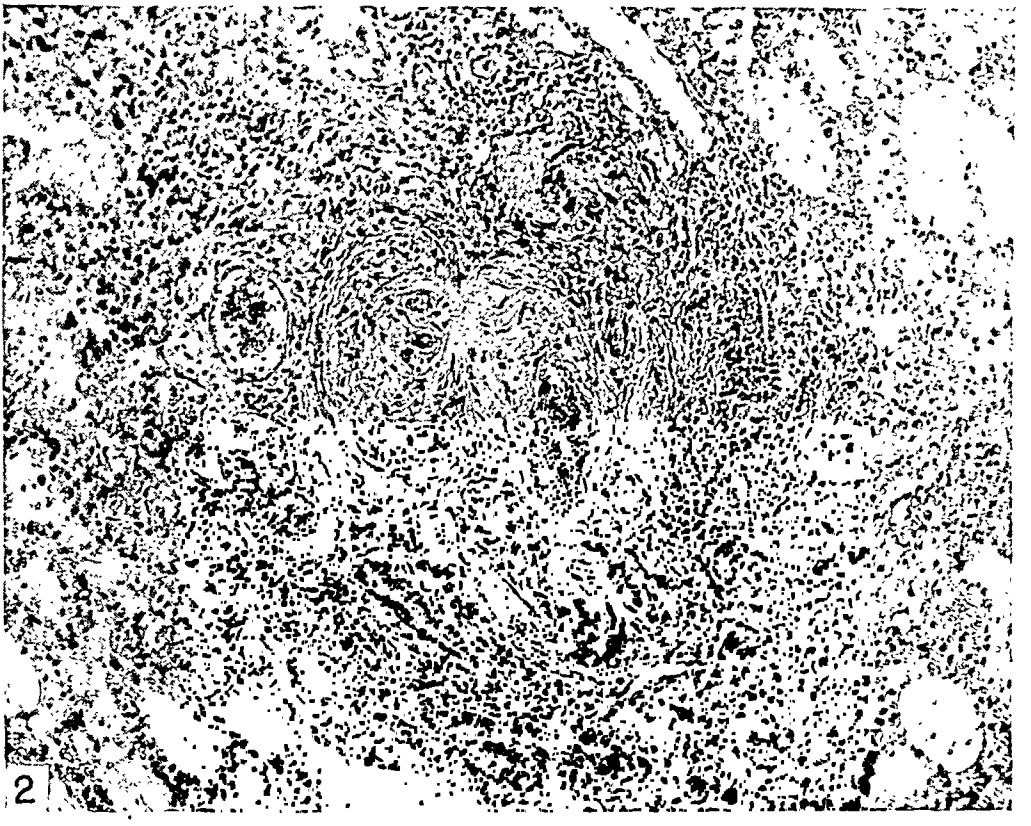
Dr. Hans Popper. "At autopsy, the pericardial sac contained 150 ml. of icteric fluid. The heart weighed 250 Gm. and the chambers of the right side revealed marked hypertrophy and dilatation. The free margin of the tricuspid valve was slightly thickened. This was not necessarily rheumatic in origin but possibly represented an effect of the hypertrophy. The left chambers were of normal shape, the mitral valve showed thickening of the free margin and the line of closure. The chordae tendineae were short, thickened and fused, suggesting a healed rheumatic lesion. The myocardium revealed periarterial spindle-shaped scars which apparently developed from Aschoff nodules. In addition, perivascular infiltration with eosinophils, histiocytes and plasma cells indicated a low-grade arteritis. The mild mitral lesion appears to have been responsible for the murmur that was heard but cannot explain the marked hypertrophy of the right heart or the clinical manifestations of cardiac failure. We are dealing, therefore, with a cor pulmonale, the etiology of which is so far problematic. The main branches of the dilated pulmonary artery, as well as their ramifications, revealed yellow, slightly elevated plaques. The pulmonary arertiosclerosis confirms the clinical suspicion of pulmonary hypertension. However, one must determine whether this is the cause or, like the cor pulmonale, the result of the increased pulmonary pressure.

"There was 200 ml. of turbid fluid in each pleural cavity. Grossly, the lungs were edematous and congested. Histologically, many bronchi were dilated.

FIG. 1. Photomicrograph of lung showing acute allergic arteritis. There is fibrinoid degeneration of part of the circumference of a small tributary of the pulmonary artery with moderate thickening of the intima and cellular infiltration consisting of histiocytes, segmented leukocytes and few lymphocytes. Dense infiltration of similar cells is found in the adventitia. × 120.

FIG. 2. Photomicrograph of lung showing chronic cicatrizing arteritis. Marked fibrous thickening of all layers of a tortuous small branch of the pulmonary artery is noted, the lumen being almost occluded by intima proliferation. The adventitial scar tissue extending up to the dilated veins reveals little cellular infiltration. In some smaller vessels, proliferation of the intima and giant cells are noted. × 120.





FIGS. 1–2

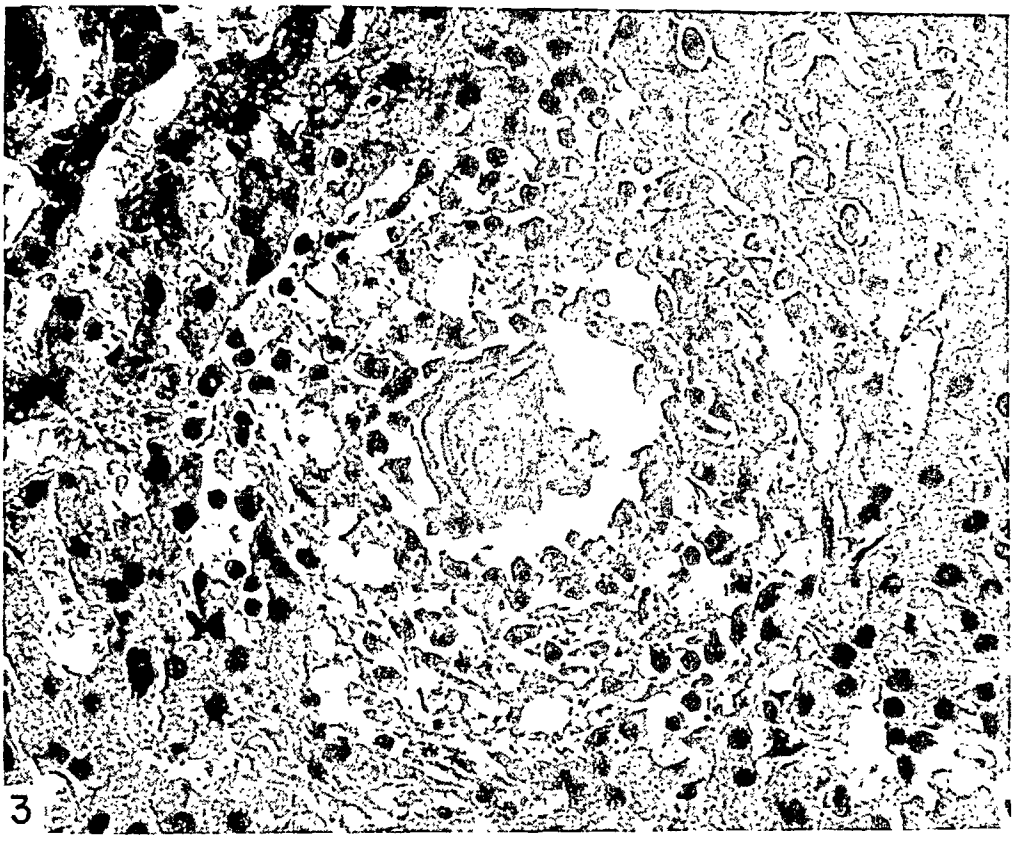
Their basement membranes were markedly thickened as is often seen in asthma. The walls of many branches of the pulmonary artery were markedly thickened. The media revealed foci of smudgy appearance, characteristic of fibrinoid degeneration. Around these foci, a dense infiltration of histiocytes, plasma cells and eosinophils extended into the periarterial tissue (Fig. 1). In some intramural necrotic foci, segmented leukocytes accumulated to produce the picture of a micro-abscess. In addition to these acute changes which suggested an allergic periarteritis nodosa, we found chronic fibrotic thickening of the arterial wall with almost complete occlusion of the lumen. These apparently were scars due to healed periarteritis (Fig. 2). The arteritic changes with the resulting obstruction of arterial blood flow caused the pulmonary hypertension and the cor pulmonale.

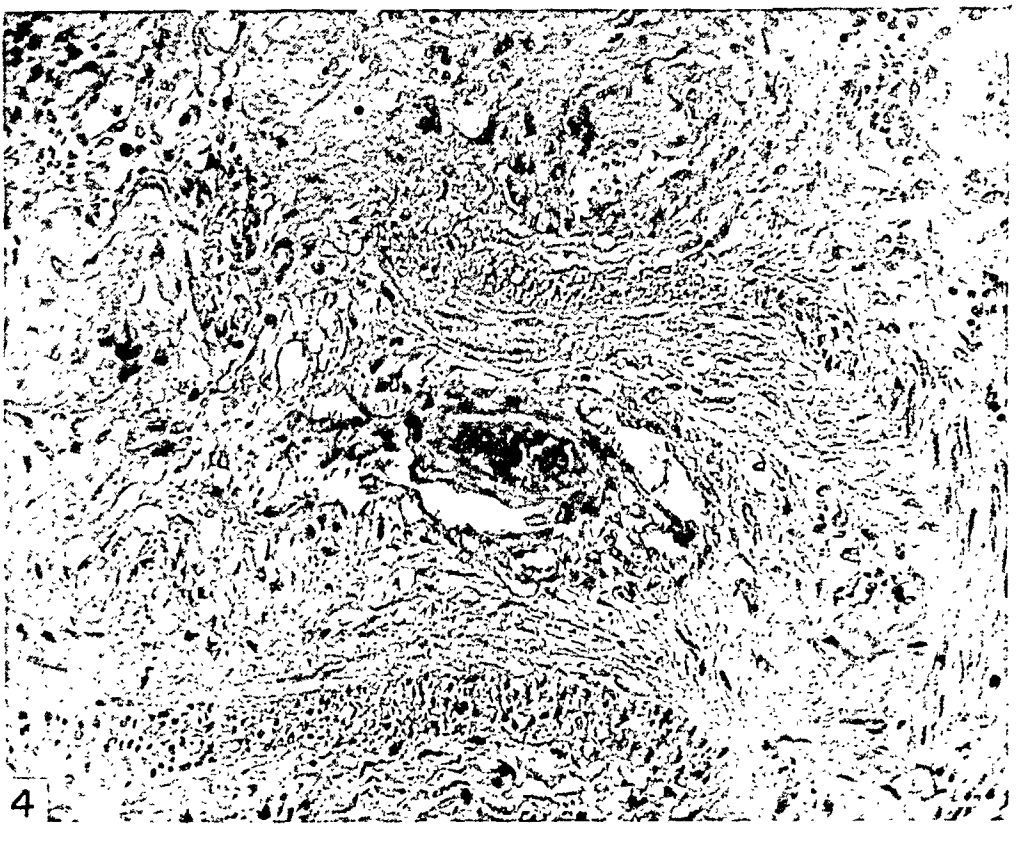
"The liver weighed 1200 Gm. and had an irregular yellow mottled surface with nodules of various sizes, up to 3 cm. in diameter. It cut with a grating sensation. On the cut surface the nodules appeared surrounded by a gray, juicy, apparently vascular connective tissue. The bed of the gallbladder was edematous. Portal, hepatic and splenic veins were patent. Histologically, the lobular architecture was greatly obscured by nodular formation which produced the picture of cirrhosis. Many of the nodules consisted of several lobules with central veins, whereas, in others, the latter were missing. The Kupffer cells were rich in a dark pigment. The centers of the lobules or nodules revealed hemorrhagic necrosis with disruption of the liver cell cords, bile casts and acidophilic staining or bile-pigment imbibition of the hepatic cells. These changes point to hepatic circulatory disturbance as well as to liver cell damage and serve to explain the jaundice and the hepatic insufficiency. In the search for the etiology of this acute hepatic damage, marked cellular infiltration of the portal triads was noted. Occasionally, in the center of the infiltrations, often surrounded by foreign body giant cells or even by pseudotubercles, irregularly shaped bodies were noted which had a refractile basophilic membrane, an organoid structure and a prominent lateral spine (Fig. 3). The appearance was characteristic of ova of *Schistosoma mansoni*.

"The adult worm, usually located in intestinal vessels, produces ova which are carried into various tissues, especially the liver and from there into the final ramifications of the portal veins. In the latter, the endothelium grows around the ova so that they finally become extravascular in location. Toxic material secreted by them leads to granuloma formation.[1] This may be followed by scarring and calcification of the ova, a feature also seen in this liver. There is a difference of opinion as to whether schistosomiasis produces cirrhosis or only periportal fibrosis.[4] Some investigators[3] have seen typical cirrhosis of the Laennec type with distortion of the lobular architecture. They consider the liver damage to be the result of a toxin produced by the parasite. This assumption would fit

FIG. 3. From section of liver. In a terminal branch of the portal vein there is an ovum of *Schistosoma mansoni* with a terminal spine. There is some proliferation of the endothelial cells and around the vessel moderate cellular infiltration, chiefly of histiocytes. × 500.

FIG. 4. Cicatrizing arteritis of branch of pulmonary artery. The lumen of a fibrotic artery contains an ovum of *Schistosoma mansoni* with very little surrounding cellular reaction. × 255.





FIGS. 3–4

the present case which exhibits a diffuse portal cirrhosis with superimposed acute hepatitis and passive congestion. The existence of portal hypertension is attested by the presence of 800 ml. of icteric ascitic fluid and by enlargement of the spleen (250 Gm.). The latter showed fibrocongestive changes and ova of Schistosoma in siderotic foci with calcium deposition. The remaining organs, including the gastrointestinal tract, contributed little of interest.

"The connection between the pulmonary arteritis and schistosomiasis remains to be explained. Allergic arteritis due to Schistosoma has been described and thorough scanning of the lung slides revealed ova of Schistosoma in some of the arteritic lesions (Fig. 4). The allergic arteritic manifestations are, therefore, not only the result of a humoral influence but, in addition, local effects of the ova must be presumed. These may possibly be associated with embolic occlusion of the lumen. In reports from Puerto Rico,[5] pulmonary involvement in schistosomiasis is not unknown. However, pulmonary hypertension as the predominant cause of death, as in this case, seems to be uncommon and only one reference to a similar case in this country was noted.[2] In Egypt, however, schistosomiasis rather frequently seems to cause a symptom complex of the nature of Ayerza's disease.[7]

"In an attempt to correlate clinical and anatomic findings it would appear that this young woman suffered from shortness of breath, ankle edema, hemoptysis and cough for several months, apparently as the result of chronic right heart failure due to pulmonary hypertension. The latter was also attested by the loud pulmonary second sound and the electocardiographic evidence of right heart strain. The pain in the chest during the last months was obviously the result of the arteritis. The cirrhosis developed over a longer period in an insidious manner without symptoms. Only during the last months did the symptoms of fever, nausea, vomiting and jaundice, and the laboratory findings of hepatic failure indicate the development of toxic manifestations with hepatic damage. The cause of death was probably a combination of cardiac and hepatic failure.

"*Pathologic diagnosis.* Schistosomiasis mansoni of liver, lungs and spleen; chronic and acute pulmonary arteritis; dilatation of pulmonary artery with arteriosclerosis; hypertrophy and dilatation of right chambers of the heart; pulmonary edema and congestion; zooparasitic cirrhosis with acute central necrosis; hydropericardium, bilateral hydrothorax and ascites; icterus; slight fibroplastic deformity of mitral value and focal myocardial fibrosis probably on rheumatic basis."

GENERAL DISCUSSION

Dr. Oscar Felsenfeld. "There is no lead in the history of this patient which would make the clinical diagnosis of schistosomiasis possible. Increased eosinophilia was absent as was the urticaria which is often observed with invasion of the lung. The liver lesions do not depend on the type of the infecting Schistosoma. Pipestem cirrhosis with enlargement of the liver is common in the Far East.[6] The most common symptoms in disease due to *Schistosoma mansoni* and *japonicum* are intestinal, especially diarrhea; in disease due to *S. hematobium*, the symptoms

are urinary. In the present case, no diarrhea was recorded. Routine stool examination would probably not have revealed the organisms because in chronic cases a special flotation technic is necessary to discover them. The most reliable means is the examination of rectal scrapings.

"In Puerto Rico, about 8 per cent of the population are infected but only about 10 per cent of infected persons have any complaints at all. The mortality in schistosomiasis depends upon the morbidity. If only one out of every 1000 people is sick, the mortality is negligible. When the morbidity reaches 10 per cent, the mortality among those infected will be as high as 25 per cent. Causes of death are lung complications or emboli to the brain and spinal cord. The skin test is of some value though it is not specific. A positive result indicates only that the person is infected with a Schistosoma or with a related organism. Therapy of Schistosoma infections is very difficult. The Egyptian form yields to antimony therapy but the mansoni and japonicum infections do not."

REFERENCES

1. Ash, J. E., and Spitz, S.: *Pathology of Tropical Disease.* Philadelphia: W. B. Saunders Company, 350 pp., 1945.
2. Clark, E., and Graef, I.: Chronic pulmonary arteritis in schistosomiasis mansoni associated with right ventricular hypertrophy; report of a case. Am. J. Path., **11:** 693–706, 1935.
3. Jaffé, R.: Was lehrt uns die Bilharzia-Zirrhose in Bezug auf die Probleme der Leber-Zirrhose? Schweiz. med. Wchnschr., **72:** 1149–1154, 1942.
4. Karsner, H. T.: Morphology and pathogenesis of hepatic cirrhosis. Am. J. Clin. Path., **13:** 569–606, 1943.
5. Koppisch, E.: Studies on schistosomiasis mansoni in Puerto Rico; morbid anatomy of the disease as found in Puerto Ricans. Puerto Rico J. Pub. Health and Trop. Med., **16:** 395–455, 1941.
6. Manson-Bahr, P. H., Editor: Manson's Tropical Diseases, a Manual of the Diseases of Warm Climates. Ed. 12. Baltimore: The Williams and Wilkins Company, 1068 pp., 1945.
7. Shae, A. F. B., and Ghareeb, A. A.: The pathogenesis of pulmonary schistosomiasis in Egypt with special reference to Ayerza's disease. J. Path., **46:** 401–424, 1938.

EDITORIALS

Neuropathology

Neuropathology as a specialty developed hand in hand with general pathology. The earliest studies and advances in neuropathology were for the most part by general pathologists and anatomists, such as Virchow, Friedreich, Golgi, von Recklinghausen, Weigert, Marchi, Waller and Robertson. In later years progress was made by men specializing in neuropathology, such as Nissl, Alzheimer, Bielschowsky, Jacob, Spielmeyer, Spiller and others. More recently neurohistologists, especially of the Spanish school, using new metallic impregnation methods, have contributed much to the cytology of the nervous system. This school was founded by Cajal and led later by his brilliant pupils Achucárro and del Rio-Hortega. Since most neurologists and some psychiatrists had extensive basic training in neurohistology and neuropathology, most of the neuropathology during the present century has been done by these two groups of physicians. Still more recently, under the stimulus of Harvey Cushing, P. Bailey and W. Penfield, neurosurgeons have been adding constantly to the understanding of surgical neuropathology. The contributions in this latter field have been to a large extent American.

From the earlier workers, until just recently, little work has been done in neuropathology by general pathologists. The present trend in some medical schools is for neuropathology to become a part of general pathology. General pathologists have been deterred from entering the field of neuropathology because of the highly specialized metallic impregnation technics advocated by neuropathologists. Another factor is the complicated nomenclature developed and employed by neuropathologists which has alienated most general pathologists. This separation of the two fields is to be very much lamented.

The move to have neuropathology become once again a part of general pathology has been stimulated just recently by the establishment of a certificate of neuropathology as a subspecialty of Pathologic Anatomy by the American Board of Pathology. Training requirements for this subspecialty have just been established. These requirements are one year of internship or one year in a laboratory, one year of training in general pathology, one year of training in clinical neurology and two years devoted to neuropathology. During this time the candidate should acquire an understanding of neuro-anatomy, neurohistology, neurophysiology, and related subjects.

The American Board of Pathology established these training requirements because it was felt that neuropathology is an inseparable division of general pathology, and thus some training in general pathology and association with general pathologists are essential. The American Association of Neuropathologists has an active membership of about seventy-five and is continuing to grow. Neuropathology is an expanding, growing specialty and frequent opportunities arise for trained neuropathologists. It is hoped that more pathologists will become interested in and contribute to this fertile field.

Rochester, Minnesota James W. Kernohan

What is the Trend?

The belief has been expressed by some that the teaching and practice of clinical pathology is being gradually turned over to departments of internal medicine in medical schools and hospitals. Although there are isolated instances which might lead one to such an impression, I doubt that there is such a general trend. If the practice of clinical pathology is drifting away from pathologists, pathologists have only themselves to blame. That some people even think there is such a trend indicates that pathologists have erred. Clinical pathology is as much a part of pathology as is pathologic anatomy. Indifference toward one is as serious a fault as neglect of the other. One may be disinterested in one or the other subspecialty but failure to recognize the existence and importance of either is not justified. Older pathologists, particularly department heads, have been especially to blame for this indifference. It is very easy to disclaim interest or enthusiasm, to deny that adequate space, personnel or necessary budget appropriations are available, and thus leave clinical pathology to any one who will take it. A convenient solution, to be sure; likewise a deplorable one; certainly not a solution that is either necessary or progressive. It should not be too great an effort or a sacrifice for a chairman or director of a department to take the responsibility for organizing a department of clinical pathology and to assume administrative charge of it. The effort to acquire facilities and personnel would be a worthwhile contribution and investment. A subdepartment might be easily created, staffed by clinical pathologists with proper delegation of responsibility. To keep clinical pathology a part of general pathology presents no real difficulties or obstacles. The failure to do so is to weaken an important specialty. Fortunately, in many ways, and despite the deplorable attitude just discussed, the actual trend appears to be quite in the opposite direction—that of strengthening clinical pathology and creating for it a necessary independence.

Detroit Osborne A. Brines

on Shortening Scientific Papers

In writing, as in speaking, it requires more effort and ability to present a message in a few words rather than in many; the shorter message is usually more effective and generally receives more attention.

Alvarez (*The Need for Shortening Scientific Papers*, Proc. Staff Meet., Mayo Clinic, **24**: 442–444, 1949) has given specific reasons and suggestions for shortening scientific papers. He advises the writer to keep introductions brief, to try to hold the reader's interest, to exclude foreign matter, to avoid long theoretical discussions, to make a good summary, to limit findings in case reports to pertinent matter, and to rework the paper several or many times before submitting it for publication.

This article by Alvarez is composed of three short pages and requires approximately four minutes to read. It should be read and its message followed by all writers of scientific papers. The formula is simple and the need is obvious.

BOOK REVIEWS

Pathology of the Nervous System. A Student's Introduction. Ed. 2. By J. HENRY BIGGART, M.D., Prof. of Pathology, Queen's University, Belfast; Pathologist to the Royal Victoria Hospital, Belfast Hospital for Sick Children. Foreword by Prof. A. Murray Drennan, M.D. 352 pp. 232 figs., 10 colored plates. $6.00. Baltimore: The Williams and Wilkins Co., 1949.

This is a volume of convenient size which has as its unique feature the effort to maintain a vital relationship between general pathology and neuropathology. Its first edition was published in 1936 as an expansion of lectures given to students preparing for examination for the Diploma in Psychiatry. The author has had long experience in general pathology followed by research in neuropathology, and throughout the book there are analogies between disease processes in the nervous tissue and in other parts of the body. The intent has been to lead the student to "apply the general principles of pathology to the lesions of the central nervous system."

Controversial material and histologic technics are omitted, while a useful chapter on the cerebrospinal fluid in disease is included. At the end of each chapter is a short bibliography of the most important references, and where experimental and epidemiologic findings are referred to briefly, the date of the work has been included.

From the point of view of the clinical pathologist, the approach is more rounded than it is in most textbooks of neuropathology. There is, for example, in the chapter on virus diseases, a brief discussion of the nature of viruses, and as each disease is considered, space is given to its epidemiology and incidence, as well as to its effects on nervous system tissue. This is done most thoroughly in the case of poliomyelitis, and here the cerebrospinal fluid findings also are included.

The chapter on brain tumors follows the classification of Cushing while the section on spinal cord tumors follows the classification of Kernohan. There are numerous especially good pictures of brain and cord showing tumors *in situ*. The photography in general is good and the placing of the illustrations is well planned.

The author reiterates in his preface to this edition that no effort has been made to turn this into a reference book. As a readable, practical piece of work, this should appeal to the general and clinical pathologist, both for his own use, and for the introduction of the subject to students and residents in a manner which is more likely to stir their interest than is a sudden plunge into the more detailed treatises on neuropathology.

Kalamazoo, Michigan HAZEL R. PRENTICE

Photoelectric Methods in Clinical Biochemistry. By G. E. DELORY, M.Sc., PH.D., Asst. Prof. of Biochemistry, University of Manitoba, Winnipeg, Canada. Formerly Biochemist, Royal Infirmary, Preston, Lancashire, England. With a foreword by Professor E. J. King, M.A., Ph.D., D.Sc. 87 pp., 21 figs. 15 s. London: Hilger & Watts, Ltd., 1949.

This is an 87 page manual of biochemical colorimetric methods, 32 pages of which are devoted to principles of photometry and a detailed description of two photoelectric photometers manufactured by the publisher of the book. The methods chosen are those in use in King's laboratory in London many of which are only slight modifications of the standard methods utilized in the United States and many of which have been replaced here by new improved methods. The descriptions of the methods are clear and concise, but explanations are either too meager or are absent altogether. The format, type and illustrations are good. There are 17 methods described, but there is no mention of bromide, salicylate, amino acid, thiocyanate, bromsulfalein, thymol turbidity and the numerous other new photometric determinations used in modern clinical laboratories. On the whole, the manual is merely a slightly more elegant version of the mimeographed book of instructions furnished by the manufacturers of any photometric instrument.

Chicago WILLIAM S. HOFFMAN

Industrial Toxicology. 2nd Edition. By ALICE HAMILTON, M.D., Assistant Professor Emeritus of Industrial Medicine, Harvard School of Public Health, Boston, Mass., and HARRIET L. HARDY, M.D., Physician to the Division of Occupational Hygiene, Mass. Department of Labor and Industries. 574 pp. $7.50. New York: Paul B. Hoeber, 1949.

Any publication by Alice Hamilton, with or without a co-author, would be a substantial addition to occupational medicine. This second edition of *Industrial Toxicology* bears little physical resemblance to the first (1934). That small first edition was one of the handsomer books of its period. This edition, much enlarged, lacks some of the niceties of format of its predecessor. Printing economy is in evidence. That mild derogation does not apply to the text.

Some prospective readers might assume from the title that this book would provide full coverage for all occupational medicine. With propriety such is not the case. Occupational medicine, from its obvious complex nature, possesses many partitions with sharp overlappings and equally sharp deviations. Industrial hygiene, preponderantly engineering, is closely allied to industrial toxicology. Clinical occupational disease activities naturally derive from industrial toxicology. In day-by-day medical activities in an industrial plant, industrial toxicology seldom dominates the requirements, but always there comes the time when industrial toxicology demands foremost consideration. Industrial medical administration, characteristic of larger corporations, is a remoter discipline; still a knowledge of industrial toxicology pervades all occupational medicine, but its domain is not exclusive. This book is a presentation of industry's chemical poisons, and is chiefly concerned with etiology and pathology. By such self-imposed limitations, the authors are enabled to avoid a voluminous work. The age-old question is raised, "What is a poison?" The classic courtroom definition leans heavily upon "chemical action". Accepting that definition, the authors would be the first to recognize that ultimately the "chemical action" of silica is responsible for silicosis. Yet, in the index neither the word "silica" nor "silicosis" appears. For sidestepping this situation and many other borderline ones—praise and praise only.

Should usefulness have been served, the authors might possibly have multiplied by twenty the number of toxic agents covered without exhausting the proved and potential harmful agents of industry. For example, it may be observed that the authors in discussing Inorganic Acids refer to some six or eight. Well known to them are at least seventy more, but their ever-present concern for the "important" fully justifies all such omissions. Even so, no fewer than 4000 industrial poisons are named, some being merely mentioned, others fully covered.

Occupational diseases never die. Some may become quite rare in occurrence and scarcely be known to newer medical generations, but their threat always remains. A virtue of this book is that it is as old as the most remote threat to any primitive workman, which probably was sunburning, and as new as the danger from radio-active isotopes.

The book's lengthy bibliographic compilation furnishes an additional quality of newness. The early books on occupational medicine, written in the United States, relied so heavily on foreign citations that it was evident that this country had no literature of its own on industrial toxicology or industrial hygiene. In this new book the majority of reference titles are in English, thus reflecting the attainment of some maturity in the contributions of the Americas to this field of medicine. If adulthood has been reached, this flows in no small measure from publications of the quality of the present one and the investigative work that lies behind them.

Detroit CAREY P. MCCORD

Dermatologie (Sammlung medizinischer Lehr- und Handbücher für Ärzte und Studierende). By J. DARIER, A. CIVATTE AND A. TZANCK. With a preface by Dr. Paul Robert, Prof. of Dermatology and Director of Dermatologic Clinic, University of Berne. 907 pp, 269 figs. 90 Fr. Bern: Hans Huber, 1949. (Grune & Stratton, New York).

The fifth edition of Darier's well known "Précis de Dermatologie" was prepared by two of his pupils, Tzanck and Civatte, and was completed after the close of the war by Civatte,

himself one of the leading French dermatologists. The present book represents the third translation into German of one of Darier's texts. It was done in Switzerland under the supervision of Professor Paul Robert by Dr. Eva Schwarz. The fact that the book is written in German will of course limit the circle of its readers in this country. The fact that it is primarily a clinical text will make it more interesting to the clinician than to the pathologist. Although it will be of greater value to dermatologists, the book should repay any medical man who reads it because it approaches diseases of the skin in true French spirit from an almost philosophic point of view and gives much more than a factual account of clinical features, diagnostic procedures and therapy. In comparison with most recent American textbooks of dermatology, the number of illustrations is small and sometimes inferior in technical quality. This, however, is not of major importance because the main value of the book is in the written word, and the space not occupied by pictures is used for a detailed, almost leisurely discussion of the various aspects of dermatology which reflects the life-long experience of the foremost exponent of the old French dermatologic school.

The greatest difficulty in presenting skin diseases in systematic fashion lies in the fact that the original morphologic nomenclature does not coincide with the newer etiologic concepts. It is, of course, not yet possible to give a complete etiologic system. The present book, instead of compromising like most authors do, separates the presentation into two parts. In the first 22 chapters, skin diseases are discussed under strictly morphologic aspects (erythema, papular eruptions, nodes, ulcers, hypertrophies, dyschromias, etc). One might say the physical diagnosis of dermatoses is presented in the form in which the student must approach a new patient. Under these headings, the various clinical and etiologic subdivisions of the lesion in question are discussed, and its pathogenesis, histopathology, and therapy are given. The second main part of the book presents an etiologic system of the dermatoses in nine chapters under such headings as parasitic dermatoses, neurodermatoses, dermatoses in diseases of the hematopoietic system, nevi, and tumors. This part unites, for instance, all the various morphologic manifestations of syphilis or of tuberculosis in one chapter and discusses the broad aspects of the disease with numerous cross references to the first part. One thought which is stressed in particular throughout the whole book is that in practice we are not dealing with diseases, but with patients, and that the predisposition of the individual patient influences the manifestations and course of the disease and the therapeutic response. A therapeutic appendix offers many formulas which will be of interest to the dermatologist. While the sulfonamides are discussed, penicillin therapy has not yet found a place in this text.

Monroe, Michigan HERMANN PINKUS

Pharmacology and Toxicology of Uranium Compounds. Edited by CARL VOEGTLIN, PH.D., Formerly Chief of the Division of Pharmacology, National Institute of Health, U. S. Public Health Service; Formerly Director of Cancer Research and Chief of National Cancer Institute; and HAROLD C. HODGE, PH.D., Professor of Pharmacology and Toxicology, School of Medicine and Dentistry, University of Rochester. Volumes I and II, 1084 pp. $10.00. New York, Toronto and London: McGraw-Hill Book Company, 1949.

These two volumes summarize the large amount of research performed during the war on the toxicity of uranium compounds. The appendix gives due credit to the large staff involved in this work. Data is present on the toxic properties of a number of uranium compounds employed in the production of atomic energy. The chemistry of these compounds, together with the analytical methods used, is presented in detail. Important studies were made on the mechanism of action of the uranium, involving the formation of protein and bicarbonate complexes with uranium, which influence its transport in the body and its excretion by the kidney. The effects of uranium on the kidney is presented in detail both from pathologic and functional studies.

In addition to the standard pharmacologic methods of estimating the lethal doses particular attention is paid to possible industrial hazards, so that safe working conditions may be established. New data is presented on the toxic properties of uranium compounds result-

ing from inhalation or by ocular and skin contacts with the agents. The soluble uranium compounds may be absorbed through the skin of experimental animals in sufficient concentration to cause death, producing the typical renal lesions. Studies are also reported on means of removing impregnated uranium compounds from the workers clothing, which is a potential source of dermal exposure. Ocular exposure, either to the dust or solutions of some compounds is also a hazard and can produce both severe local reactions and systemic poisoning. A great deal of data is given on the inhalation of various uranium dusts and recommendations for respiratory protective equipment are made. The theory of tolerance to uranium is discussed and the role of citrate in tolerance is described. Other chapters describe the effects of uranium on enzymes and protein, and report the distribution, retention and excretion of the compounds. The detection of over-exposure to uranium, by the measurement of catalase in urine, is described in detail, as are the other methods and technics used in the various studies. A section of the book is devoted to the pharmacology and toxicology of fluorine and hydrogen fluoride.

Although many of the biological data were obtained from small groups of animals, the results have been studied statistically and the conclusions drawn are valid. There tends to be some overlapping of data in various chapters, but considering the volume of the studies, and the fact that the data is summarized after most of the investigators have left the project, it would seem to be unavoidable. These volumes provide important data on the action of uranium compounds from the point of view of fundamental drug action and industrial toxicity. Although the older literature is summarized, most of the text describes new unpublished studies.

Detroit VICTOR A. DRILL

Shock and Allied Forms of Failure of the Circulation. By H. A. DAVIS, M.D., C.M., F.A.C.S., Associate Professor of Surgery, Director, Division of Surgery, Graduate School of Medicine, College of Medical Evangelists, Los Angeles Division. 595 pp., 55 figs. $12.00. New York: Grune & Stratton, 1949.

This monograph is one of the most significant publications in modern medical writing. It is so because of the importance of its subject matter and the scholarship brought to it. Shock is a term which has lost its original exclusive association with trauma, but stands for a set of phenomena familiar to and feared by every practicing physician and surgeon. The problem of shock has had brought to it considerable thought and experiment. Clear concepts have been delayed by inaccurate observation and undue emphasis on single phenomena, as well as by ignorance. Davis' broad conception of shock is "a state involving the entire organism, characterized by a generalized impairment of the circulation, and caused by any form of stress or injury which reduces the output of blood from the left ventricle of the heart to a level below that needed for normal cellular function and metabolism." To marshal the evidence for this concept, to appraise it critically, and to construct a rational program of prevention and resuscitation is the burden of this book.

The first two chapters are on history, classification, and diagnosis of shock and shock-like states. Traumatic shock and hemorrhage are considered for their impact on the whole organism. Pathogenesis, physiologic and biochemical changes, and pathology are treated in great detail. There follow chapters on the effects of anoxia and hypoxia in man and lower animals, irreversible shock, medical and obstetrical shock. Circulatory failure is studied in its association with toxemias and infections, diseases of the liver, and adrenal glands, the nervous system, disturbances of the thorax and its contents, burns and other forms of thermal injury, and anesthesia. A provocative chapter on the circulatory "weakling", and an admirable one on the treatment of shock and allied forms of circulatory failure complete the book.

This monograph is well balanced in construction and writing. It contains a wealth of experimental and clinical evidence, much of it conflicting, which is appraised with mature judgment. It therefore stands as a valuable reference work. The subject matter pertains to the laboratory and to the patient whether sick or injured. It can be recommended to every-

one who has to do with the biological phenomena associated with shock, whether in the laboratory or the sick room.

Dearborn, Michigan GAYLORD S. BATES

Advances in Internal Medicine. Vol. 3. Edited by WILLIAM DOCK, M.D., Long Island College of Medicine, Brooklyn, N.Y. and I. SNAPPER, M.D., The Mount Sinai Hospital, New York. Associate Editors: T. R. Harrison, M.D., C. S. Keefer, M.D., W. T. Longcope, M.D., G. R. Minot, M.D., J. M. Steele, M.D. 444 pp., 76 figs. $8.50. New York: Interscience Publishers, 1949.

The book is the third of a series, a fourth of which is in preparation, compiling the devel opment in internal medicine of the last decade. The fourteen authors who have prepared the text of this volume have each been leading contributors to the development of their respective topics.

The subjects covered are varied: Use of British Anti-lewisite, Streptomycin, Penicillin, Anti-thyroid compounds, New Therapeutic Agents for Neurologic Conditions, Plasma Fractionation, Histoplasmosis, Hemolytic Anemias, Value of Enzyme Determinations in Diagnosis, and the Mechanism of Acclimatization to Heat. The comprehensive bibliographies supplied by the authors for those who wish a more detailed discussion are particularly welcome.

Most of the sections are direct, clear and concise. A number of articles are particularly good. Dr. Janeway's discussion of Plasma Fractionation brings together the extensive wartime research aimed at the production of purified serum albumin as a convenient, stable, safe, easily-transported blood substitute for the initial treatment of shock and the important uses of the many by-products of this production; namely, anti-hemophilic globulin, thrombin, fibrinogen products, gamma globulins, and more potent isohemagglutinins for typing purposes. Dr. Eagle's discussion of the extremely rapid development and application of penicillin strikes a nice balance between the fundamental research and clinical applications. New developments including the procaine penicillins have revised somewhat previously held concepts. Dr. Feldman and Dr. Hinshaw have conservatively summarized the widespread application of streptomycin to tuberculosis control. One might desire, however, a little more detailed discussion of the toxicity both to the patient and to those administering it.

Dearborn, Michigan HAROLD J. KULLMAN

Cardiovascular Disease. By LOUIS H. SIGLER, M.D., F.A.C.P., Attending Cardiologist and Chief of Cardiac Clinic, Coney Island Hospital. 527 pp., 149 figs. $10.00. New York· Grune and Stratton, 1949.

A large amount of carefully selected, useful material has been brought together in this volume. Several preliminary chapters deal with the incidence, physiology, and pathology of the cardiovascular system and of specific altered states in general. These chapters provide an excellent brief review of the fundamentals of disease. The author devotes considerable space to methods of physical diagnosis in general, and to each specific disease in particular. It is refreshing to find clinical acumen emphasized more than mechanical devices in the recognition and control of cardiovascular disease. The several chapters dealing with problems in specific diseases are broad in scope and well organized. Special methods of diagnosis, such as the x-ray and the electrocardiogram, are treated only as part of the diagnostic procedure and with due regard to their limitations. The author is to be commended for his sensible recommended treatment. On controversial questions he presents both sides of the subject and only occasionally expresses his own opinion. This is especially evident in his chapter on Heart Failure which assumes increasing interest with the development of new methods for investigation. This important subject might have been given greater attention, but it is brief and clear, as is the book in general. The book is well illustrated both by charts and pictures, and is indexed. Each chapter has a well selected bibliography of the most important contributions. The book withal would make a useful addition to any physician's library.

Detroit N. E. CLARKE

SELECTED ABSTRACTS

Ueber eine seltene Herz-Gefässmissbildung: Fehlen des Aortenbogens. W. HERZOG. Frankf. Ztschr. f. Path., **59:** 454–460, 1948.

Herzog reports a rare type of congenital cardiac anomaly in a six-day old female infant. The aortic arch was absent, as was the brachiocephalic trunk. There was also a large subaortic interventricular septal defect, as well as a patent foramen ovale. The ductus arteriosus was still open, and the left subclavian artery took origin at the entrance of the ductus Botalli into the descending aorta. The author points out that, considering the clinical picture and embryologic processes, the left subclavian artery had to take its origin where it did in the presence of agnesis of the aortic arch.

Fort Wayne, Indiana S. M. RABSON

Untersuchungen zur Pathologie des Pankreas im ersten Lebensjahr unter besonderer Berücksichtigung des Bindegewebes. G. ULE. Frankf. Ztschr. f. Path., **59:** 359–380, 1948.

Ule reviews well the accumulated data concerning size and weight of the pancreas in the neonatal and infant states, and reports on 72 premature babies and infants up to one year of age. He concludes that weight and length of the organ and its relation to body weight and length varies too much to permit its use in determining age and maturity of children. Incidentally, a reprint of Rössle and Roulet's "Mass und Zahl in der Pathologie" would be found of service to most pathologists.

Connective tissue is the predominant tissue in premature infants and in the early months of life. The author regards the finding of hematopoietic centers in the early weeks as evidence of the embryonal function. Three cases are cited without clinical evidence of pancreatic disease in two of which the organ was the seat of severe phelgmonous inflammation. The third was diagnosed as "secretory stasis following outflow obstruction", although the point of obstruction was not looked for or found. This case satisfies the criteria of fibrocystic disease described by Andersen and by Farber.

S. M. RABSON

Allergene aus Brucella abortus Bang. W. MOSIMANN. Schweiz. Ztschr. f. Path. u. Bakt., **12:** 362–379, 1949.

In the introduction to his work on "Allergens of Brucella abortus Bang", Mosimann (Hygienic-Bacteriologic Institute, University of Berne) reviews the types of allergens already isolated and the methods of their recovery. He attempted to improve the allergens for the cutaneous test for undulant fever in man. Four substances were prepared and tested on mice and rabbits for toxicity and on rabbits for immunologic status: full extract, nucleoprotein fraction, gluco-lipoido-amino acid complex (endotoxin) and polysaccharide.

The polysaccharide, which demonstrated the properties of a hapten, was the only one that was nontoxic to mice, while all were harmful to rabbits in greater concentration, the endotoxin being most toxic and the polysaccharide least. It was possible, however, to achieve dilutions which were not toxic. Antigen effectiveness (in rabbits) was most marked when using living organisms. Followed by endotoxin, nucleoprotein and full extract, the polysaccharide was ineffective. Antiserum production was best with the gluco-lipoid and with the polysaccharide, although it was noted with the other two. Sensitized rabbit underwent cutaneous testing with the different extracts, and it was found that the polysaccharide was best suited to demonstrate hypersensitivity by skin test.

S. M. RABSON

Tissue Proteins and Carcinogenesis. II. Electrophoretic Studies on Serum Proteins during Carcinogenesis due to Azo Dyes. H. A. COOK, A. C. GRIFFIN, and J. M. LUCK. J. Biol. Chem., **177:** 373–381, 1949.

Feeding of some azo dyes produces cancer of the liver in rats. In the work of E. C. Miller and J. A. Miller (Cancer Research, **7:** 468, 1947), these dyes were shown to be bound by

hepatic proteins. In the present paper the effect of the dyes on serum proteins was studied by means of electrophoresis. Rats fed the highly carcinogenic compound, m'-methyl-p-dimethylaminoazobenzene, showed, after two weeks, a decrease in the albumin concentration and an increase in the gamma-globulin fraction. Alpha- and beta-globulin fractions were not significantly affected. These changes in serum proteins were interpreted as nonspecific, depending on liver damage. However, a relationship between carcinogenicity of azo dyes and their effect on serum proteins was suggested by the fact that the less active dye p-dimethylaminoazobenzene produced changes of a lesser degree than the methyl derivative mentioned above, and that the relatively noncarcinogenic compound, azobenzene, caused only minimal changes. A carcinogen, 2-acetylaminofluorene, not belonging to the azo compounds, failed to affect the serum proteins within the period of observation.

Chicago KURT STERN

Osmotic Resistance of Erythrocytes from Patients with Carcinoma. T. H. WILSON and W. F. LANDMESSER, JR. Cancer, **2:** 503–509, 1949.

The fragility of red blood cells was studied by means of "an improved method for the detection of osmotic abnormalities of erythrocytes" (M. H. Jacobs, D. R. Stewart, W. J. Brown, and L. J. Kimmelman. Am. J. M. Sc., **217:** 47–52, 1949). In this technic red cells are suspended in hypotonic solutions of sodium chloride, and in glycerol, respectively; photoelectric readings indicate the degree of hemolysis under constant conditions. The blood of 90 cancer patients was studied. The primary site of the tumor influenced the results: in carcinoma of the stomach and carcinoma of the colon, about three-fourths of the patients showed increased resistance of erythrocytes; in carcinoma of the breast less than 40 per cent showed this change. The authors did not observe a definite relationship between red cell fragility and presence of metastases. They considered a possible role of anemia in producing an increased osmotic resistance in patients with cancer but, on the basis of their present material, they could not arrive at a definite conclusion.

KURT STERN

Chymotrypsin in Cancer. M. B. SHIMKIN and H. R. BIERMAN. Proc. Soc. Exper. Biol. and Med., **71:** 250–252, 1949.

Ineffectiveness of Chymotrypsin Therapy in Malignancy. P. M. WEST. Proc. Soc. Exper. Biol. and Med., **71:** 252–253, 1949.

For some time, reports on cancer cures attributed to injection of chymotrypsin have been current (See J. A. M. A., **193:** 93, 1949.). Therefore, it is important to take note of the unequivocally negative results presented in these two papers. The first, by Shimkin and Bierman, is based on a study of 10 patients with cancer, the second by West, records observations on 15 patients with cancer and on 2 groups of mice with transplanted sarcomas. In the animals, tumor growth in treated and untreated groups proceeded in a parallel manner. In none of the cancer patients did the enzyme produce any subjective or objective effect on the disease, nor did autopsy findings disclose any gross or histologic changes attributable to the treatment. Severe anaphylactoid reactions occurred in some of the treated patients. Hence, no benefit, but, rather possible serious damage, can be expected from the use of chymotrypsin in cancer.

KURT STERN

Heterologous Ocular Transplantation as a Practical Test for Cancer. J. A. SCHILLING, A. C SNELL and B. V. FAVATA. Cander, **2:** 480–490, 1949.

The ability of a transplant to grow in the anterior chamber of the eye of the guinea pig has been described as a unique feature distinguishing autonomous malignant tumors from other heterologous tissues (H. S. N. Greene). As the result of a considerable number of investigations by Greene and other workers with this method, a significant contribution has been made to the biology of cancer. The authors of the present paper reviewed this work, including several studies on heterotransplantation of tumors, into the eye of guinea pig,

going back as far as 1873. The primary purpose of their own investigation was to determine whether this method could be useful as a diagnostic laboratory procedure. Thirty-six histologically proved malignant tumors were tested; a total of 218 guinea pigs was used for ocular transplantations. Only in 8 of the 36 tumors (in 29 eyes of 20 animals) was there heterologous growth. In 19 instances tissue cultures were prepared from the same tumors used for the heterotransplantation, and growth of the cultures was obtained in 15 cases. In view of the large number of human malignant tumors which failed to grow in the eye of the guinea pig, the authors conclude that the method cannot be used clinically for diagnosis. The usefulness of the method, however, in instances of successful growth lies in the insight which it affords into the biology of cancer, particularly with regard to growth-potential and morphology.

KURT STERN

Proteolytic Enzyme Inhibitors of the Blood in Relation to Neoplastic Diseases. P. M. WEST and J. HILLIARD. Ann. West. Med. and Surg., **3:** 227–234, 1949.

A previous publication of the authors (Proc. Soc. Exper. Biol. and Med., **71:** 169–172, 1949) presented methods for determining the inhibitory effect of human serum on the enzymes, chymotrypsin and rennin. In the course of investigating 2000 cases, it was noted that a variety of physiologic and pathologic factors changed the normal pattern of these enzyme-inhibitors.

In the present paper West and Hilliard studied the concentrations of inhibitors of chymotrypsin and rennin in serum of cancer patients. In general, neoplastic disease is accompanied by elevation of both inhibitors in the serum. This change cannot be utilized for diagnostic purposes since it is nonspecific. However, a significant correlation was found to exist between the relative proportions of the two inhibitors and the rate of growth of the malignant tumors. High concentrations of chymotrypsin-inhibitor and low concentrations of renninhibitor were observed in serum of patients with fulminating course of disease. Inversely, low values of chymotrypsin inhibitor and high values of rennin inhibitor were associated with apparent clinical control of cancer. Such a phenomenon (rise of rennin inhibitor and drop of chymotrypsin inhibitor) became manifest, for instance, after effective x-ray therapy; the enzymatic pattern changed prior to clinical evidence of tumor-regression. Since radiation therapy was ineffective and failed to produce such changes in the enzyme inhibitors, and since check-tests also enabled one to recognize reactivation of the tumor process, valuable prognostic information can be derived from the tests. Enzyme changes similar to those found after effective radiation treatment, were also observed in patients responding favorably to palliative chemotherapy (nitrogen mustards, urethane, hormones). Hence, the authors propose to utilize the quantitative study of the enzyme-inhibitors in the evaluation of chemotherapy of cancer, and, in general, to employ the test as a means of prognosticating the course of neoplastic disease.

KURT STERN

The Histochemical Demonstration of Esterase. M. M. NACHLAS and A. M. SELIGMAN. J. Nat. Cancer Inst., **9:** 415–425, 1949.

This report deals with the presence of several carboxylic esterases (choline esterase, lipase, and nonspecific esterase) in animal tissues and their demonstration by a new staining method.

Tissues fixed in chilled acetone were embedded in paraffin and stained at pH 7.8 in a solution containing beta naphthyl acetate and alpha naphthyl diazonium naphthalene-1,5-disulfonate. Approximately 60 per cent of the original esterase activity was lost in the fixation and embedding. Esterase activity was highest in normal lung, liver, pancreas, and kidney tissue of the rat and dog.

New Orleans BEN FISHER

A New Method for the Histochemical Demonstration of Acid Phosphatase. A. M. SELIGMAN and L. H. MANHEIMER. J. Nat. Cancer Inst., **9**: 427–434, 1949.

This continuation of previous work by the authors introduces a new method by which both alkaline and acid phosphatases may be demonstrated by differential staining of a single tissue section.

Using diazotized calcium alpha naphthol, at pH 5, an insoluble brown-red pigment was produced which could be incubated at room temperature for as long as 24 hours without excessive decomposition of the compound. The tissues were fixed in acetone. Tissues studied included normal prostate and neoplastic prostate gland after stilbesterol therapy and/or castration.

BEN FISHER

Zur pathologischen Anatomie des Darmbrands. H. SCHOEN. Frankf. Ztschr. f. Path., **59**: 525–550, 1948. *Die tiefe, "Unspezifische, Enterogene" Wandentzündung des Verdauungschlauchs (Unter besonderer Berücksichtigung der 1946/47 gehauft aufgetretenen Enteritis Gravis).* K. KLOOS. Frankf. Ztschr. f. Path., **60**: 1–57, 1949.

Early in the summer of 1946, the north German coast was visited by an epidemic of gastrointestinal disease which did not lessen in severity until the end of the following year. From time to time, up to 1945, the condition was encountered in operative specimens. The pathology of the malady forms the subject for two papers, the second by Kloos being more comprehensive and dealing with 50 autopsied cases. Of these, 45 autopsies were performed by Kloos in the city of Kiel. Schoen, on the other hand, reports only 4 cases, all from the region of Göttingen where the disease did not achieve epidemic proportions. The following abstract is based on the published conclusions of both authors.

The disease, according to Kloos, is nonspecific and the causative agent is characterized as "enterogenous". Any part of the digestive canal may be attacked; the stomach and esophagus are least involved and the upper jejenum, lower ileum, cecum and splenic flexure of the colon are most prominently diseased. There are two forms of the disease, acute and chronic. All 50 fatal cases in Kiel were acute; 12 died within 3 days, 15 in the second half of the first week and 17 during the second week.

Anatomically, the manifestations of the malady are protean, and Kloos compares the deep mural lesions in the intestines to those of appendicitis with its varied morphologic phenomena. The inflammation is usually erosive and pseudomembranous and later necrotizing and ulcerative. In all early cases there is marked hyperemia and edema. In the small intestine lesions first appear on the summits of the folds and in the villi, while in the colon both longitudinal and transverse folds are affected. Scattered foci are recognized in the stomach, while a similar state or a diffuse condition is found in esophagus and rectum.

In the second phase of the disease necrosis is the chief feature, first identified in the upper segments and on the transverse folds. The destructive process usually ceases deep in the mucosa or in the adjacent third of submucosa. In the third stage there is maceration and sloughing of necrotic tissue. Cellular reaction is usually slight about the areas of necrosis and ulceration, while in the adjacent vessels a variety of changes from edema to fibrinoid necrosis, transudation of plasma, diapedesis of red cells and thrombosis is found. Kloos thinks that the vascular alterations are significant in the pathogenesis, and are not unlike those in dysentery. When the vessels are much changed, intraluminal intestinal hemorrhage appears; hemorrhage most often takes place during maceration of necrotic lesions. Occasionally, necrosis is deep, usually takes place when the disease is intermittently progressive, and leads to perforation (14 per cent, second week). Peritonitis, at first of sterile character and usually confined to the involved segments, is found in a little less than half the cases. The exudate is fibrinous or serofibrinous and inhibits free or open perforation into the abdominal cavity.

Sometimes, says Kloos, there is mechanical narrowing of the intestinal lumen by muscular or nervous disturbance of motility produced, in turn by mural exudation and rigidity and by loss of musculature. In the stomach severe dilatation is the result. Healing is fol-

lowed by flat scars, often slate-gray, with loss of the usual rugal pattern; stenotic scars are seldom recognized and represent former walled-off perforation.

Schoen is impressed by mucosal necrosis which may be seen in various stages of development. He reports larger and smaller necrotic foci in the inner muscular layer. Leukocytic infiltration serves to limit areas of necrosis. The cells usually encountered are lymphocytes, plasma cells and histiocytes. Like Kloos, Schoen emphasizes intestinal edema, especially of the submucosa, capillary and venous dilatation and engorgement, hemorrhage and thrombosis. He, too, records mural swelling and necrosis of vessels, especially of arteries.

The lower ileum, according to Kloos, may occasionally be the seat of a chronic granulomatous process, which takes root in the primary circumscribed variety, perhaps when the general immunologic status is better. Kloos suggests that on the one hand, local defense is better in the caudal segments of the small intestine, and, on the other, the mechanical irritation of intestinal content delays healing and promotes the development of a rich reparative process. This 'chronic' granulomato-fibroblastic form is morphologically identical with the 'chronic plastic phlegmon' of the intestine, with so-called terminal ileitis and the so-called 'inflammatory tumor' of the colon," writes Kloos. Schoen, on the other hand, quite vehemently rejects any identification of Crohn's disease with the malady he reports. He calls upon Muir, and Illingworth and Dick, and does not indicate whether he himself had cases of terminal ileitis for comparison.

No causative organism has thus far been found, and Kloos suggests that the infection is complex, perhaps on a viral basis. In an addendum there is mention of the finding by various workers of aerobic fusobacteria (Bacillus fundilo-formis), of Bacillus enterotoxicus type "f", of a microorganism producing beta-toxin.

Secondary bacterial growth is responsible for pyelophlebitic liver abscesses, embolic abscesses in lung and myocardial provoked by intestinal aerobic microorganisms and, sometimes, gas-gangrene infection. Distant thrombosis is infrequent, and is usually responsible for local complications, most often late in the disease. Confluent bronchopneumonia is the immediate cause of death in many cases. The splenic pulp does not respond, except when there are extra-gastrointestinal complications. Liver and kidneys are represented by insignificant uncharacteristic lesions.

Although all ages were susceptible, the greatest mortality was found after the 40th year of life, and largely among men. The prognosis apparently was better in women than in men.

Severe acute cases, as in dysentery, writes Kloos, resulted from "a general toxic collapse, the lesion being seen in various mucosae with production of necrotic-hemorrhagic processes, for example, in throat, urinary bladder and vagina." Schoen thinks that the morphologic findings justify the "guess . . . that an allergy-conditioned mechanism can be the basis, whereby one thinks especially of the similarity with the Sanarelli-Shwartzmann phenomenon." He points to primary circulatory disturbances as of special importance in the production of tissue changes.

S. M. Rabson

OBITUARIES

Bonnelle William Rhamy

On February 11, 1874 Dr. Rhamy was born in Fort Wayne, Indiana. He graduated from the Fort Wayne College of Medicine in 1898 and returned there in 1902 as a professor of Physiology. In 1905 he became Bacteriologist for the city of Fort Wayne.

Dr. Rhamy's initiative and devotion to his specialty are reflected in his outstanding work in the development of Clinical Pathology. He was a charter member of the American Society of Clinical Pathologists, one of the founders of the Indiana State Pathological Association, and the founder of the Fort Wayne Medical Laboratory. He was also a member of the Indiana State Council, American Medical Association, College of American Pathologists, American Association for the Advancement of Science, National Geographical Society, American Heart Association, Fort Wayne Academy of Science, Indiana Medical Advisory Board No. 4, Venereal Disease Control Commission of the Indiana State Medical Association, and the Fort Wayne Chamber of Commerce. At various times, he served as pathologist at the Defiance (Ohio) Hospital, Sacred Heart Hospital, Garren, Wells County Hospital, Bluffton, Parkview Hospital, Plymouth, Woodlawn Hospital, Rochester, Lutheran, Methodist, and St. Joseph's Hospitals. Dr. Rhamy's contribution to these many organizations stand as evidence of his unselfish spirit.

Dr. Rhamy died of arteriosclerotic heart disease and diabetes mellitus on May 26, 1949. His passing will long be mourned by a multitude of friends and colleagues. He is survived by his wife and daughter.

Robert Koritschoner

Dr. Robert Koritschoner was born in Vienna in 1884, and died on November 15, 1948 in Kansas City, Missouri. He received his medical degree in Vienna and subsequently came under the influence of Richard Paltauf, with whom he was associated for a number of years.

Dr. Arthur Steindler, Professor of Orthopedic surgery at the University of Iowa, was instrumental in bringing Dr. Koritschoner to America. Shortly after his arrival he went to Iowa City and remained there in the field of Pathology for three years. In 1925, he came to Kansas City to accept the position of pathologist at St. Joseph Hospital. During this period he was also associated with the Department of Pathology at the Kansas City General Hospital. When Menorah Hospital was opened in September, 1931, he was invited to become its pathologist and Director of Laboratories, a position which he held until his death. Dr. Koritschoner became a member of the American Society of Clinical Pathologists in 1935.

Exerting a profound influence on the elevation of the standards of medicine and surgery at Menorah Hospital and on the practice of pathology in Kansas City, Dr. Koritschoner carried out original investigations in rabies, encephalitis, connective tissue structure, fat embolism, paradoxic embolism, irradiation effect on cancer of the breast, and pathologic physiology of the gallbladder.

Dr. Ferdinand Helwig of Kansas City paid Dr. Koritschoner the following tribute: "The influence of Robert Koritschoner was of immeasurable value in raising the science of hospital pathology to its present high level in Kansas City. . . He was in every respect a doctor's doctor and his scientific counsel and diagnostic skill were sought eagerly and relied upon unhesitatingly by all of his medical confreres."

Howard J. Maldeis

Howard J. Maldeis was born in Baltimore, Maryland on September 20, 1879. He received his medical degree from the University of Maryland in 1903 and devoted himself to pathology from the time of his graduation until his death. In 1907 he became Clinical Pathologist at the University of Maryland Hospital and remained in this position until 1921. During these years he was Assistant Professor of Histology and Embryology in the Medical School,

and Chief of the Science Laboratory in the University of Maryland School of Dentistry.

From 1917 to 1939 he was the City Autopsy Physician for Baltimore, and from 1921 until 1939 he engaged in the private practice of pathology. For years he fought strenuously for the abolition of the coroner's system in his state, and when this was finally accomplished in 1939, he became the first Chief Medical Examiner for the State of Maryland, which position he retained until his death.

Dr. Maldeis was a member of the Medical and Chirurgical Faculty of the State of Maryland and a Fellow of the American Medical Association. He was also a Diplomate of the American Board of Pathology, a Founding Fellow of the College of American Pathologists, a past president of the Baltimore City Pathological Society, and a Fellow of the American Society of Clinical Pathologists since 1924.

Dr. Maldeis died January 15, 1949, from carcinoma of the pancreas. His wife and two children survive him.

Walter Clinton Jones

Dr. Jones was born in Chicago, May 2, 1874. He was graduated from Northwestern University Medical School in 1902 and was certified by the American Board of Pathology in 1939. A charter member of the American Society of Clinical Pathologists, Dr. Jones was a pioneer in the development of several clinical hospital laboratories in Birmingham, Alabama. He was a founding member of the Alabama Association of Pathologists and had the honor of serving as its first president in 1946. With much enthusiasm he attended each succeeding gathering of the group.

Dr. Jones was possessed of wide interests which, among others, included music and biologic sciences. He was an inspiring teacher. He exhibited a dogged determination to master the problems which confronted him. He was forthright in his views and dependable in his actions. Dr. Jones did much to further the study and practice of pathology in Alabama and must be remembered as among the first personally to carry this specialty into remote regions of this state.

Herman Spitz

Dr. Herman Spitz was born in Hungary on June 29, 1885, and as an infant, was brought to this country by his parents who settled in Cleveland, Ohio. Later they moved to Nashville, Tennessee. Dr. Spitz attended Vanderbilt University Medical School, from which he was graduated in 1912. The year following was spent with Dr. William Litterer in bacteriologic laboratory work. From 1914 to 1919, Spitz engaged in postgraduate work in pathology at Cornell Medical School and at Harvard.

From 1914 to 1930, Dr. Spitz served as pathologist at the Woman's Hospital in Nashville. During this period he established his private laboratory for bacteriology and pathology in the Doctors Building. He served as Bacteriologist in charge of the Nashville City Health Department from August, 1940 until the time of his death. He was a member of the Tennessee State Medical Society and a Fellow of the American Medical Association. Dr. Spitz was a charter member of the American Society of Clinical Pathologists, a member of the Executive Committee from 1923 to 1928, and served as State Counselor from 1932 to 1937. Dr. Spitz died on February 4, 1949.

Harold A. Thompson

Dr. Harold A. Thompson was born in 1883 in Pennsylvania, received his medical degree from the University of Southern California in 1906, and practiced in San Diego from 1907 to his death, July 31, 1948. He was one of the first to specialize in clinical pathology in California.

For a long period before World War I, he was the only physician-anesthetist in San Diego. He was City and County Bacteriologist from 1907 until 1947. In addition he directed a private laboratory in clinical pathology. Dr. Thompson was a hard worker but entered into

recreational activities just as enthusiastically. Illness in his last years lessened his time for both work and recreation but did not diminish his ardor for either. He is survived by his wife, Elsa, two daughters, and five grandchildren.

Dr. Thompson became a member of the American Society of Clinical Pathologists in 1925.

William Edward Kelly

Dr. Kelly was born in Middletown, New York in 1876. He graduated from Hahnemann, Philadelphia, Pennsylvania in 1904. The Orange County Medical Society elected Dr. Kelly to their membership on May 6, 1924 and a year later he became a member of the American Society of Clinical Pathologists. He was affiliated with Goshen (N. Y.) Hospital and St. Frances Hospital in Port Jervis and also served for some time as pathologist at Middletown State Homeopathic Hospital. On November 24, 1948, Dr. Kelly died of cerebral hemorrhage.

During the years of his membership in the Society, his work on various committees to which he was assigned proved his sterling qualities. While he held no office, his wise counsel was of great value.

Frank Anthony Pugliese

Dr. Frank Anthony Pugliese was born October 13, 1908 in Walston, Pennsylvania. He attended Temple University Medical School and was graduated in 1935. Dr. Pugliese interned at Allegheny General Hospital, Pittsburgh, took his residency in clinical medicine and pathology, and engaged in postgraduate work in clinical pathology and internal medicine at Tulane University during the years 1944, 1945, and 1947. He was a member of the American Medical Association and the American Society of Clinical Pathologists. From 1939 to the time of his death, Dr. Pugliese served as pathologist at the Adrian Hospital, Punxsutawney, Pennsylvania.

Dr. Pugliese died on November 17, 1948 of acute leukemia. He is survived by his wife and two children.

INDEX OF SUBJECTS

Abstracts, selected
Authors
Adams, W. S., *et al.*, Multiple myeloma, 584
Ayres, W. W., Staining nucleoli of cells, 590
Bailey, W. S., and Williams, A. G., Balantidium infection, 654
Baker, H., and Blood, W. L., Gram stain, 589
Bassen, F. A., *et al.*, False positive trichina precipitation tests, 856
Berman, L., *et al.*, Blood and bone marrow in cirrhosis, 1052
Bernoulli, R., Ein Nickhautcarcinom bei einem Hirsch, 587
Boemke, Fr., Der plötzliche Tod aus natürlicher Ursache bei Soldaten, 184
Boltjes, T. Y. K., Bacterial flagella, 254
Botsztejn, Ch., and Zollinger, H. U., Metastasierender hypernephroider Nierentumor, 586
Brass, K. A., Die Eiweissstoffwechselstörungen des Plasmocytomkranken, 588
Calvey, G. L., Pseudohemophilia, 255
Cameron, G. R., *et al.*, Effect of castration on action of some barbiturates, 254
Cardelle, G., *et al.*, Weltmann reaction, 254
Chen-Jen, C., and Zia, S. H., Fixed rabies virus, 584
Cocchi, U., and Stueckelberger, P., Sippentafel mit gehäuftem Krebsvorkommen, 587
Cohen, S. M., Accidental intra-arterial injection of drugs, 186
Collins, D. H., Bullet embolism, 253
Conley, C. L., *et al.*, Circulating anticoagulant, as cause of hemorrhagic diathesis, 589
Cooper, T., and Watkins, C. H., Sternal aspiration, 1052
Cosgriff, S. W., Heparin and dicumoral anticoagulant therapy, 255
Crepea, S. B., and Cooke, R. A., Mechanism of dermatitis venenata, 585
DeAngelis, G., Fungus, action on mouse cancer, 857
Duguid, J. B., Thrombosis in pathogenesis of aortic atherosclerosis, 187
Dukes, C. E., Rectal cancer, 1059
Farmer, L., Histamine refractoriness, 584
Fischer, H., Entzündliche Gehirnerkrankungen im frühen Kindesalter, 188
Fisher, J. W., Optimal hemolysis level, 585
Fitzpatrick, J., and Tompsett, S. L., Riboflavine and Nicotinic acid, 1058
Foot, N. C., and Humphreys, G. A., Prognosis of renal tumors, 183
Fowler, N. O., Blood coagulation in postoperative state, in congestive heart failure and thrombophlebitis, 1056
Franco, S., and Wolman, M., "Cellule de Gaucher", 186
Frantz, V. K., *et al.*, Radioactive iodine studies of functional thyroid cancer, 252
Frei, R., Ueber die histopathologischen Veränderungen am Dünndarm, 185
Friedmann, I., Cystic pneumatosis, 1059
Gardini, G. F., Ginecomastia con degenerazione cancerigna, 586
Gedgoud, J. L., and Tollman, J. P., Arteriolosclerosis, 190
Green, M. N., and Friedgood, C. F., Furicin, 857
Hall, B. E., and Campbell, D. C., Vitamin B_{12} therapy in pernicious anemia, 394
Herzog, W., Ueber eine seltene Herz-Gefässmissbildung, 1161
Higginson, J. F., and Clagget, O. T., Esophagus disruption, 460
Humble, J. G., Mechanism of petechial hemorrhage formation, 654
Hunziker, H., Post-traumatische Osteosklerose der Patella, 188
Hynes, M., Iron reserve of a normal man, 1059
Jarrold, T., and Vilter, R., Hematologic observations in chronic hepatic insufficiency, 856

Kästli, P., Die Lebensfähigkeit von *M. tuberculosis*, 588
Kauer, G. L., Leukemias, treatment, 253
Kidd, P., Elution of incomplete type of antibody in acquired hemolytic anemia, 1058
Kidder, G. W., *et al.*, Purine metabolism, 858
Koch, W., Die Wirkung verschiedener Kieselsäureformen im sensibilisierten Tierorganismus, 1055
Kollbruner, F., Gutartiger Riesenzelltumor mit maligner Entartung, 585
Lowbeer, L., Brucellotic osteomyelitis, 1056
Mann, F. D., and Hurn, M., Blood coagulation, 190
Marinelli, L. D., *et al.*, Factors involved in experimental therapy of metastic thyroid cancer with I^{131}, 252
Moloney, W. C., *et al.*, Acquired afibrinogenemia in pregnancy, 654
Moore, G. E., Radioactive diiodofluorescein, 191
Mosimann, W., Allergene aus *Brucella abortus* Bang, 1161
Nachlas, M. M., and Seligman, A. M., Histochemical demonstration of esterase, 1163
New York Academy of Sciences, Amino acid excretion, 189
Nicod, J. L., Les lésions vasculaires dans le poumon silicotique, 1053
Nieset, R. T., *et al.*, Red blood cell volume, determination with radioactive phosphorus, 583
Olwin, J. H., One- and two-stage prothrombin methods, 1057
Parkin, T. W., *et al.*, Antiheparin compounds in thrombocytopenic purpura, 190
Pearse, A. G., Russell bodies and Kurloff bodies, 1058
Perr, H. M., Sickle cell anemia with special reference to diagnostic parameter, 583
Petermann, M. L., *et al.*, Electrophoretic studies on plasma proteins in neoplastic disease, 191
Pfeiffer, C. A., and Allen, E., Producing cancer in rhesus monkeys, 191
Quick, A. J., *et al.*, Coagulation time in hemorrhagic diseases, 590
Quick, A. J., and Stefanini, M., Prothrombin level of blood, 394
Ratnoff, O. D., Proteolytic enzyme in human plasma, 460
Robertson, J. S., and Smith, K. V., Experimental dissection of aortic media, 187
Rose, H. M., *et al.*, Differential agglutination of sheep erythrocytes, 189
Rosenthal, R. L., Opalescence of serum after x-irradiation, prognosis, 1060
Roskam, J., *et al.*, Vascular fragility test, 590
Schilling, J. A., *et al.*, Heterologous ocular transplantation, 1053
Schoen, H., Zur pathologischen Anatomie des Darmbrands, 1164
Seligman, A. M., and Manheimer, L. H., Histochemical demonstration of acid phosphatase, 1163
Sellek, A., *et al.*, Kline cardiolipin reaction, 254
Shimkin, H. R., and Bierman, H. R., Chymotrypsin in cancer, 1162
Shimkin, M. B., *et al.*, Blood histamine, 1057
Sibley, J. A., and Lehninger, A. L., Aldolase, 857
Smith, T. R., Coagulation defect, 190
Spitz, S., Histological effects of nitrogen mustard on tumors, 253
Stout, A. P., and Kenway, F. R., Plasma cell tumors of upper air passages and oral cavity, 856
Sturgis, C. C., Etiology and treatment of hematological disorders, 583
Tompsett, S. L., Neutral 17-ketosteroids, 1058
Uehlinger, E., Die akute Silikose des sarganser Beckens, 1053
Ule, G., Pathologie des Pankreas im ersten Lebensjahr, 1161
Verhagen, B. A., Calcium-formol gel reaction, 856
Von Vaczy, L., Beiträge zur Rolle des Follikelhormons, 587
Webster, S. H., Heinz body phenomenon, 1053
Wegelin, C., Die Natur der sogenannten Myoblastentumoren, 185
Weiner, M., *et al.*, Platelet adhesiveness, 460
West, P. M., Ineffectiveness of chymotrypsin therapy in malignancy, 1162

West, P. M., and Hilliard, J., Proteolytic enzyme inhibitors, 1163
Williams, A. W., and Mackey, J. P., Determination of sickle cell trait, 1052
Wilson, T. H., and Landmesser, W. E., Osmotic resistance of erythrocytes, 1162
Wintrobe, M. M., and Huguley, C. M., Nitrogen mustard therapy, 252
Zwarenstein, H., Orthotolidine hydrochloride test, 1059
Abstracts, selected
Titles
Accidental intra-arterial injection of drugs, 186
Acid phosphatase, histochemical demonstration of, 1163
Afibrinogenemia in pregnancy, 654
Agglutination, differential, of sheep erythrocytes, 189
Aldolase, in serum and tissues of tumor-bearing animals, 857
Allergene aus *Brucella abortus* Bang, 1161
Amino acid excretion, symposium, 189
Anticoagulant as cause of hemorrhagic diathesis, 589
Antiheparin compounds in essential thrombocytopenic purpura, 190
Aortic athersclerosis, pathogenesis of, 187
Aortic media, factors in experimental dissection of, 187
Arteriolosclerosis in nine year old child, 190
Bacterial flagella, function and arrangement, 254
Balantidium infection in dog, 654
Beiträge zur Rolle des Follikelhormons, 587
Benign pelvic tumors, 184
Blood and bone marrow in cirrhosis, 1052
Blood coagulation in congestive heart failure and thrombophlebitis, 1056
Blood histamine, in leukemia and erythremia, 1057
Brucellotic osteomyelitis, 1056
Bullet embolism, 253
Calcium-formol gel reaction, quantitative, 856
Cancer, heterologous ocular transplantation as test for, 1053
Cancer in rhesus monkeys, carcinogenic hydrocarbons and estrogens, 191
Castration, effect of, on action of barbiturates, 254
Chymotrypsin in cancer, ineffectiveness of, 1162
Coagulation defect produced by nitrogen mustard, 190
Coagulation time in study of hemorrhagic diseases, 590
Complement, relation to blood coagulation, 190
Darmbrands, Pathologie des, 1164
Dermatitis venenata in guinea pig, mechanism of, 585
Dünndarm, über die histopathologischen Veränderungen am, 185
Eiweissstoffwechselstörungen des Plasmocytomkranken, 588
Electrophoretic studies in neoplastic disease, 191
Erythrocytes in acquired hemolytic anemia, elution of incomplete type of antibody from, 1058
Esophagus disruption, complete, 460
Esterase, histochemical demonstration of, 1163
Furacin, action in transplanted fibrosarcoma, 857
Gaucher, cellule de, 186
Gehirnerkrankungen im kindesalter, über *distruiersende entzündliche*, 188
Ginecomastia con degenerazione cancerigna, 586
Gram stain, studies on, 589
Heinz body in erythrocytes, 1053
Hematologic disorders, etiology and treatment, 583
Hematologic observations in chronic hepatic insufficiency, 856
Hemolysis level, optimal, in complement-fixation test, 585
Heparin and dicumarol, effect on sedimentation rate, 255
Herz-Gefässmissbildung, über eine seltene, 1161
Histamine refractoriness, experiments on, 584
Iron reserve of normal man, 1059
Ketosteroids (17-) in urine, determination of, 1058
Kieselsäureformen, die Wirkung verschiedener, im sensibilisierten Tierorganismus, 1055

Kline cardiolipin reaction in congenital syphilis, 254
Leukemias, treatment of, 253
Multiple myeloma, 584
M. tuberculosis, die Lebensfähigkeit von, 588
Myoblastentumoren, die Natur der sogenannten, 185
Nickhautcarcinom, ein, bei einem Hirsch, 587
Nierentumor, metastasierender hypernephroider, 586
Nitrogen mustard for Hodgkin's disease, lymphosarcoma, etc., 252
Nitrogen mustard, histological effects on human tumors, 253
Orthotolidine hydrochloride test for blood in urine, 1059
Osmotic resistance of erythrocytes, from carcinoma, 1052
Osterosklerose der Patella, post-traumatische, 188
Pankreas, Pathologie des, im ersten Lebensjahr, 1161
Pathologic classification in prognosis of renal tumors, 183
Petechial hemorrhage formation, mechanism, 654
Plasma cell tumors of upper air passages and oral cavity, 856
Platelet adhesiveness, 460
Pneumatosis, cystic, of large intestine, 1059
Poumon silicotique, le, relations avec la tuberculose, 1053
Proteolytic enzyme inhibitors of blood in relation to neoplastic diseases, 1163
Proteolytic enzyme in human plasma, 460
Prothrombin level of blood, experimental, 394
Prothrombin methods in dicumarol therapy, one- and two-stage, 1057
Pseudohemophilia, 255
Purine metabolism in Tetrahymena, relation to malignant cells in mice, 858
Rabies virus in brain of guinea pig fetus, 584
Radioactive diiodofluorescein in diagnosis and localization of brain tumors, 191
Radioactive iodine studies of functional thyroid cancer, 252
Rectal cancer, surgical pathology of, 1059
Red blood cell volume with radioactive phosphorus, 583
Russell bodies and Kurloff bodies, 1058
Sickle cell anemia, laboratory diagnosis of, 583
Sickle cell trait, determination of, using reducing agent, 1058
Silikose, die akute, des sarganser Beckens, 1053
Sippentafel mit gehäuftem Krebsvorkommen, 587
Staining nucleoli in fresh benign and malignant tissues, 590
Sternal aspiration in diagnosis of malignant lymphomata, 1052
Streptothrix felix D. A., action on mouse cancer, 857
Thyroid cancer, metastatic, experimental therapy, with I^{131}, 252
Tissue proteins and carcinogenesis, 1161
Tod, der plötzliche, aus natürlicher Ursache bei Soldaten, 184
Trichina precipitin tests, false positive, in infectious mononucleosis, 856
Vascular fragility test, inconstancy and variability of, 590
Vitamin B_{12} in pernicious anemia, 394
Weltmann reaction in infantile tuberculosis, 254
X-irradiation, opalescence of serum, as prognostic sign of death, 1060
Acid-fast bacilli
concentration of, 269
in sputum, 688
Acid-fastness of chemically induced inclusion bodies, 608
Actinomycosis, pathologic and bacteriologic study, 201
Adhesiveness of blood platelets
measurement of, 701
significance of, 814
Adrenal gland, cortical necrosis in diphtheria and malaria, 899
Adsorption levels of prothrombin-free plasmas, 1071
Agglutinability of red cells, in pyogenic infections, 782
American Board of Pathology, list of certified pathologists, February Supplement
Aminopterin in treatment of leukemia, 119

Amyloid, atypical, solitary tumors of, 1097
Animals, universal serologic reaction with serum of, 361
Antibodies
of third order, 134
titration of, 265
Antigens, cardiolipin, in tests for syphilis, 156
Anti-Rh agglutinins, clinical significance, 435
Antithrombin activity, in plasma, 1024
Antitryptic activity in human plasma, 448
Apparatus
instrument to level tissue in paraffin embedding, 897
laboratory water connections, 499
microdetermination of cholesterol, 1076
multiple puncture for vaccination, 492
paraffin tissue-block trimmer, 1070
pipetting instrument for serologic use, 693
prism mirror, 96
sedimentation tube support, 298
serum total base and sodium, 659
sheep blood, collection and dispensing, 496
tissue dehydrating and embedding, 1061
Argyrosis, excretion of silver in, 668
Bacteriology
actinomycosis; pathologic and bacteriologic study; 21 cases; 201
blood culture bottle, 697
Clorox and trisodium phosphate, for acid-fast bacilli in sputum, 688
colored cotton plugs, 98
concentrating acid-fast bacilli, 269
divided culture plate, 289
Dubos' medium, 67
identification of salmonellas, 106
inclusion bodies induced by lead and bismuth, acid-fastness of, 608
Maxted's filtrate for extracting carbohydrates, 880
M. tuberculosis from sputum or gastric washings, 91
penicillin-resistant staphylococci, 1083
primary isolation of dermatophytes, 95
solid-and-liquid medium, 697
streptomycin sensitivity of *M. tuberculosis*, 67, 76
trichrome stain for culture or smear, 898
tubercle bacilli, culture of, from gastric juice, 685
BCG vaccination, apparatus, 492
Berylliosis, beryllium determination in diagnosis of, 229
Bile, bromsulphalein in presence of, 699
Biochemistry
antitryptic activity in plasma, 448
beryllium determination, 229
bromsulphalein in presence of hemolysis, turbidity, and bile, 699
bromsulphalein tests, error in, 700
caronamide, effect on phenosulfonphthalein, 381
correlation of morphologic liver changes and clinical tests, 710
co-thromboplastin assay, 861
flame photometry, 461
Folin-Ciocalteu's phenol reagent, 297
ketosteroids (17-), significance and methods of determination, 424
lecithinase activity in splenic dyscrasias, 615
microdetermination of cholesterol, 1076
mucopolysaccharide content in skin in myxedema, 442
nomograph for icterus index, 884
para-aminosalicylic acid blood levels, 886
phosphatases in blood, 167
protein in edema fluid, 488
prothrombin-free plasmas, electrophoretic analysis, 1071
prothrombin, two-stage method, 471
recent advances in knowledge of prothrombin, 41
serum lipids
after nephrectomy, 962
in mercuric chloride poisoning, 962
serum total base and sodium, conductivity measurements of, 659
silver, excretion of, in argyrosis, 668
thromboplastin from rabbit lung, 71
urinary sugar test for blind, 500
Xenopus laevis, for pregnancy tests, 284
Biopsy
cytohistologic diagnosis of material aspirated from stomach, 328
obtaining tissue from stomach with sponge, 891
use of sponge in microtechnic, 92
Blood banking
production of therapeutic fractions of blood serum, 10
Rh antibody titrations, accuracy of, 1032
survey of operation of blood banks, 35
ultrafiltration and carbon dioxide, effect on antithrombin, 1024
Blood cells, peripheral electron microscopy of, 217

Blood coagulation, co-thromboplastin assay, 861
Blood culture bottle, use of solid-and-liquid medium, 697
Blood donor sets, silicone-treated needles in, 695
Blood factor rh^{w}, 621
Blood phosphatases, 167
Blood platelets
adhesiveness of, in thromboembolism and hemorrhagic disorders, 814
counting, 1113
counts in rabbits, 1122
in thromboembolism and hemorrhagic disorders, 701
Blood transfusions, use of group-specific substances in Group O, 536
Blood volume, estimation of, 483
Book reviews
Authors
Addis, T., Glomerular Nephritis, 459
Albright, F., and Reifenstein, E. C., Parathyroid Glands and Metabolic Bone Disease, 992
American Public Health Association, Diagnostic Procedures for Virus and Rickettsial Diseases, 192
Anderson, W. A. D., Pathology, 193
Arnow, L. E., Introduction to Physiological and Pathological Chemistry, 593
Ashford, M., Trends in Medical Education, 1048
Behrendt, H., Diagnostic Tests for Infants and Children, 994
Benbrook, E. A., and Sloss, M. W., Manual of Veterinary Clinical Parasitology, 257
Biggart, J. H., Pathology of Nervous System, 1156
Birkeland, J., Microbiology and Man, 850
Blackwood, W., *et al.*, Atlas of Neuropathology, 596
Cancer Committee of Iowa State Medical Society, Diagnosis and Treatment of Cancer, 198
Carter, C. F., Microbiology and Pathology, 195
Chang, K., Studies on Hookworm Disease in Szechwan Province in West China, 993
Coley, B. L., Neoplasms of Bone and Related Condition, 655
Conn, H. F., Current Therapy, 1949, 854
Crile, G., Jr., Practical Aspects of Thyroid Disease, 855
Crohn, B. B., Regional Ileitis, 995
Dameshek, W., and Estren, S., The Spleen and Hypersplenism, 397
Dameshek, W., and Taylor, F. H. L., George R. Minot Symposium on Hematology, 1049
Darier, J., *et al.*, Dermatologie, 1157
Davis, H. A., Shock, 1159
Delory, G. E., Photoelectric Methods in Clinical Biochemistry, 1156
Dock, W., and Snapper, I., Advances in Internal Medicine, 1160
Favre, M., Le Foie Vasculaire, 197
Fowler, W. M., Hematology for Students and Practitioners, 997
Fried, B. M., Bronchogenic Carcinoma and Adenoma, 256
Gershenfeld, L., Urine and Urinalysis, 396
Glick, D., Techniques of Histo- and Cytochemistry, 592
Goldblatt, H., The Renal Origin of Hypertension, 595
Gradwohl, R. B. H., Clinical Laboratory Methods and Diagnosis, 193
Hepler, O. E., Manual of Clinical Laboratory Methods, 398
Herbut, P., Surgical Pathology, 592
Hevesy, G., Radioactive Indicators, Their Application in Biochemistry, Animal Physiology and Pathology, 596
Himsworth, H. P., The Liver and Its Diseases, 395
Hoskins, M. M., and Bevelander, G., Outline of Histology, 397
Jones, H. W., *et al.*, Blakiston's New Gould Medical Dictionary, 1051
Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, 455
Karsner, H. T., Year Book of Pathology and Clinical Pathology, 1050
Lejwa, A., Symposia on Nutrition of the Robert Gould Research Foundation, 257
Libánský, J., Diagnostické Nabodávání Mízních uzlin, Nadorů a Dužinových Orgánů, 594
Long, P. H., A-B-C's of Sulfonamide and Antibiotic Therapy, 198
Lyons, W. R., and Woodhall, B., Atlas of Peripheral Nerve Injuries, 993

Mackie, T. J., and McCartney, J. E., Handbook of Practical Bacteriology, 591
Málek, I., and Wagner, V., Mikrobiologické Vyšetřovací Methody a Stručná Diagnostika v Praxi, 595
Martin, L., and Hynes, M., Clinical Endocrinology for Practitioners and Students, 996
Maximow, A. A., and Bloom, W., A Textbook of Histology, 656
Means, J. H., *et al.*, Thyroid Function as Disclosed by Newer Methods of Study, 457
Mégroz, R. L., Ronald Ross, Discoverer and Creator, 454
Meleney, F. L., Clinical Aspects and Treatment of Surgical Infections, 594
Meulengracht, E., Acta Haematologica, 657
Neuhof, H., Venous Thrombosis and Pulmonary Embolism, 594
Nickerson, W. J., Biology of Pathogenic Fungi, 456
Pack, G. T., Cancer of the Esophagus and Gastric Cardia, 397
Parpart, A. K., Chemistry and Physiology of Growth, 992
Pepper, O. H., Medical Etymology, 1051
Pick, J., *et al.*, Terezin Očima Hygienika, 595
Ponder, E., Hemolysis and Related Phenomena, 458
Rappaport, F., Rapid Microchemical Methods for Blood and CSF Examinations, 996
Roberts, G. F., The Rhesus Factor, 195
Romeis, B., Mikroskopische Technik, 1050
Ronchese, F., Occupational Marks and Other Physical Signs, 192
Scheinker, I. M., Neurosurgical pathology, 196
Sicher, H., Oral Anatomy, 593
Sigler, L. H., Cardiovascular Disease, 1160
Smith, D. T., *et al.*, Zinsser's Textbook of Bacteriology, 591
Smith, L. W., and Gault, E. S., Essentials of Pathology, 398
Sturgis, C. C., Hematology, 395
Thomas, E. W., Syphilis: Its Course and Management, 995
Trincao, C., Sindroma Anemico do Kala-Azar, 459
Turner, C. D., General Endocrinology, 997
van Rooyen, C. E., and Rhodes, A. J., Virus Diseases of Man, 656
Vaughan, W. T., Practice of Allergy, 196
Voegtlin, C., and Hodge, H. C., Pharmacology and Toxicology of Uranium Compounds, 1158
Walther, H. E., Krebsmetastasen, 850
Westermark, N., Roentgen Studies of Lungs and Heart, 198

Titles
A-B-C's of Sulfonamide and Antibiotic Therapy, 198
Acta Haematologica, 657
Advances in Internal Medicine, 1160
Atlas of Neuropathology, 596
Atlas of Peripheral Nerve Injuries, 993
Biology of Pathogenic Fungi, 456
Blaskiston's New Gould Medical Dictionary, 1051
Bronchogenic Carcinoma and Adenoma, 256
Cancer of Esophagus and Gastric Cardia, 397
Cardiovascular Disease, 1160
Chemistry and Physiology of Growth, 992
Clinical Aspects and Treatment of Surgical Infections, 594
Clinical Endocrinology for Practitioners and Students, 996
Clinical Laboratory Methods and Diagnosis, 193
Current Therapy 1949, Latest Approved Methods of Treatment for the Practicing Physician, 854
Dermatologie, 1157
Diagnosis and Treatment of Cancer, 198
Diagnostic Procedures for Virus and Rickettsial Diseases, 192
Diagnostic Tests for Infants and Children, 994
Diagnostické nabodávání Mízních Uzlin, Nádorů a Dužinových Orgánů, 594
Essentials of Pathology, 398
Experimental Immunochemistry, 455
General Endocrinology, 997
George R. Minot Symposium on Hematology, 1049

Glomerular Nephritis, 459
Handbook of Practical Bacteriology, 591
Hematology, 395
Hematology for Students and Practitioners, 997
Hemolysis and Related Phenomena, 458
Industrial Toxicology, 1157
Introduction to Physiological and Pathological Chemistry, 593
Krebsmetastasen, 850
Le Foie Vasculaire, 197
Liver and Its Diseases, 395
Manual of Clinical Laboratory Methods, 398
Manual of Veterinary Clinical Parasitology, 257
Medical Etymology, 1051
Microbiology and Man, 850
Microbiology and Pathology, 195
Mikrobiologické Vyšetřovací Methody a Stručná Diagnostika v Praxi, 595
Mikroskopische Technik, 1050
Neoplasms of Bone and Related Condition, 655
Neurosurgical Pathology, 196
Occupational Marks and Other Physical Signs, 192
Oral Anatomy, 593
Outline of Histology, 397
Parathyroid Glands and Metabolic Bone Disease. Selected Studies, 992
Pathology, 193
Pathology of the Nervous System, 1156
Pharmacology and Toxicology of Uranium Compounds, The, 1158
Photoelectric Methods in Clinical Biochemistry, 1156
Practical Aspects of Thyroid Disease, 855
Practice of Allergy, 196
Radioactive Indicators. Application in Pathology, 596
Rapid Microchemical Methods for Blood and CSF Examinations, 996
Regional Ileitis, 995
Renal Origin of Hypertension, 595
Rhesus Factor, The, 195
Roentgen Studies of Lungs and Heart, 198
Ronald Ross, Discoverer and Creator, 454
Shock and Allied Forms of Failure of Circulation, 1159
Sindroma Anemico do Kala-Azar, 459
Spleen and Hypersplenism, The, 397
Studies on Hookworm Disease in Szechwan Province in West China, 993
Surgical Pathology, 592
Symposia on Nutrition of Robert Gould Research Foundation, 257
Syphilis: Its Course and Management, 995
Techniques of Histo- and Cytochemistry, 592
Terezín Očima Hygienika, 595
Textbook of Histology, A, 656
Thyroid Function as Disclosed by Newer Methods of Study, 457
Trends in Medical Education, 1048
Urine and Urinalysis, 396
Venous Thrombosis and Pulmonary Embolism, 594
Virus Diseases of Man, 656
Year Book of Pathology and Clinical Pathology, 1050
Zinsser's Textbook of Bacteriology, 591
Bone formation in heart, 974
Bone marrow granulomas in herpes zoster, 981
Bromsulphalein tests
 error in, 700
 presence of hemolysis, turbidity and bile, 699
Bronchial adenoma with cancerous transformation, 836
Bronchial secretions
 a cytohistologic study, 323
 tumor cells in, 320
Bronchogenic carcinoma, cytologic diagnosis of, 765
Burned bodies, medicolegal examination of, 599
Cancer, symposium on cytologic diagnosis of, 301
Carbon dioxide, effect on antithrombin activity of plasma, 1024
Carcinoma
 of cervix, progression from "precancer" stage, 770
 of prostate, cytologic diagnosis of, 315
 of rectum, *Schistosoma japonicum* infection, 985
Carcinogenesis, chemical, pathology of, 1095
Cardiolipin antigen
 in Kolmer complement-fixation test, 25
 in tests for syphilis, 156

Cardiolipin-lecithin antigen, in Kline test, 274
Caronamide, effect of, on excretion of phenosulfonphthalein, 381
Case reports
acute hemolytic anemia associated with cold agglutinins, 630
asymptomatic *Schistosoma japonicum* infection, 985
bone formation in heart, 974
bronchial adenoma with cancerous transformation, 836
dilatation of acini of pancreas in hyperemesis of pregnancy, 846
epidermoid cyst of spleen, 558
Gaucher's disease in a Negro, 570
granulomas in bone marrow in herpes zoster, 981
interstitial cell tumor of testis, 1039
leiomyoma angiomatosum of mesentery, 639
leiomyosarcoma of stomach, 966
malignant pheochromocytoma and hypertension, 243
massive stilbamidine therapy of multiple myeloma, 634
neurinoma of small intestine, 827
paracolon endocarditis, 840
primary
primary splenic neutropenia, 48
replacement transfusion, after reaction from incompatible blood, 1131
sarcoidosis, 725
simulated homicide, obstructive laryngeal papilloma, 565
Cerebrospinal fluid
interpretation of findings in, 16
Kline test for syphilis in, 274
Cervix, progression from "precancer" stage to carcinoma, 770
Cholesterol, microdetermination of, 1076
Clinicopathologic conferences
chronic pulmonary fibrosis, etiology unknown, 643
disseminated lupus erythematous, 177
fat embolism, 388
hemochromatosis and carcinoma of liver, 789
lymphosarcoma of stomach, 576
schistosomiasis mansoni of liver, lungs and spleen, chronic and acute pulmonary arteritis, 1146
Clorox, acid-fast bacilli from sputum, 688
Coagulation mechanism, 1016
Cold agglutinins associated with acute hemolytic anemia, 630
Collecting multiple blood specimens, technic of, 293
Collodial gold reagent, preparation, 86
Complement-fixation test
Kolmer, 25
quantitative measurement, 141
with pooled sheep cells, 152
Concentrating acid-fast bacilli, 269
Concentration of protozoan cysts for iron hematoxylin staining, 84
Conductivity assembly for serum total base and sodium, 659
Correlation of morphologic liver changes and clinical tests, 710
Co-thromboplastin assay, in blood coagulation, 861
Cotton plugs, for culture mediums, 98
Culture diagnosis of fungus disease, 865
Cytologic diagnosis
of cancer from pleural and peritoneal fluids, 309
of carcinoma of prostate, 315
of uterine cancer by examination of vaginal and uterine secretions, 301
Cytohistologic diagnosis of material aspirated from stomach, 328
Cytohistologic study of bronchial secretions, 323
Dermatophytes, primary isolation of, 95
Detection of acid-fast bacilli, by fluorescent microscopy, 72
Diffusely enlarged uterus, 544
Dilatation of acini of pancreas in hyperemesis of pregnancy, 846
Dioxane, for paraffin embedding, 291
Diphtheria, experimental cortical adrenal necrosis in, 899
Distribution of erythrocyte fragility, 259
Divided culture plate, 289
Dubos' medium, 67
Dynamic equilibrium, 999
Editorials:
Dr. Giordano, Our True Physician, 51
Howard T. Karsner, 990
Neuropathology, 1154
On shortening scientific papers, 1155
Pathways in pathology, 796
Round cell infiltration, 393
Training of clinical pathologists, 653
What is the trend? 1154
Electron microscopy of peripheral blood cells, 217

Electrophoretic analyses, of prothrombin-free plasmas, 1071
Endamoeba histolytica, growth of virus of poliomyelitis in, 1135
Endocarditis, paracolon, 840
Epidermoid cyst of spleen, 558
Erythrocytes
fragility, distribution, 259
improved suspension medium, 88
washed and unwashed, in titration of antibodies, 265
Esophageal varices, occurrence in routine autopsies, 554
Flame photometry, for sodium and potassium in plasma and urine, 461
Fluorescent microscopy, for acid-fast bacilli, 72
Foam cell plaques in intima of myometrial arteries, 30
Folic acid antagonist in chronic leukemia, 127
Folin-Ciocalteu's phenol reagent, 297
Fractionation of agglutinins, blocking antibodies and cryptagglutinoids, 134
Frozen section technic, 873
Fungi
culture diagnosis, advances in, 865
microslide for culture of, 278
Gastric juice, culture of tubercle bacilli from, 685
Gastric washings, recovery of *M. tuberculosis*, 91
Gaucher's disease in a Negro, 570
Granulomas in bone marrow in herpes zoster, 981
Group-specific substances, use in group O transfusions, 536
Hashimoto's and Riedel's strumas and lymphadenoid goiter, 750
Heart, bone formation in, 974
Hematology
abnormal agglutinability of red cells in pyogenic infections, 782
acute hemolytic anemia associated with cold agglutinins, 630
adhesiveness of blood platelets, 701, 814
blood platelets, 1113, 1122
blood volume, 483
electron microscopy of blood cells, 217
erythrocyte fragility, distribution of, 259
Hodgkin's disease
effect of nitrogen mustard on, 372
sternal marrow in, 929
Köhler illumination for reticulocyte count, 883
lecithinase activity in splenic dyscrasias, 615
leukemia, chronic, treatment with aminopterin, 119, 127
new methylene blue as reticulocyte stain, 895
photographic recording of hemolysis, 676
platelets, texchrome staining of, 681
primary splenic neutropenia, 48
replacement transfusion for reaction from incompatible blood, 1131
sedimentation tubes, support, 298
stilbamide therapy in multiple myeloma, 634
suspension medium, improved erythrocyte counts, 88
thin blood films, 98
Hemolysis
bromsulphalein in presence of, 699
photographic recording of, 676
Hemolytic streptococci, Maxted's filtrate, 880
Hemolytic system of complement-fixation test, use of pooled sheep cells, 152
Hemorrhagic disorders, adhesiveness of blood platelets in, 701
Herpes zoster, bone marrow granulomas in, 981
Hodgkin's disease
nitrogen mustard in, 372
sternal marrow studies, 929
Human blood serum, production of therapeutic fractions of, 10
Hyperemesis of pregnancy, dilatation of pancreatic acini in, 846
Hypertension and malignant pheochromocytoma, 243
Icterus index, nomograph for, 884
Inclusion bodies induced by ingestion of lead and bismuth, 608
Incompatible blood, treatment of reaction from transfusion with, 1131
Infectious mononucleosis, test for, 665
Interstitial cell tumor of testis, 1039
Intra-uterine anoxia, degenerative liver changes, 801
Ion-exchange resins, removal of salts with, 10
Iron hematoxylin staining of protozoan cysts, 84
Irradiation of myometrium, presumptive sign of, 30
Ketosteroids (17-), significance and methods of determination, 424

Kline test of cerebrospinal fluid for syphilis, 274
Knife sharpener, microtome, 1089
Köhler illumination for reticulocyte counts, 883
Kolmer complement-fixation test, cardiolipin antigen in, 25
Laboratory water connections, 499
Lecithinase activity in splenic dyscrasias, 615
Leiomyoma angiomatosum of mesentery, 639
Leiomyosarcoma of stomach, 966
Lepromatous leprosy, universal serologic reaction with serum of, 408
Leukemia
folic acid antagonist in, 127
treatment with aminopterin, 119
Lipo-adrenal extract, effect on ionic balance, 907
Liver changes, correlation of morphology and clinical tests, 710
Liver, degenerative changes from intrauterine anoxia, 801
Lung carcinoma, needle aspiration in diagnosis of, 235
Lymphadenoid goiter, Hashimoto's and Riedel's strumas, 750
Malaria
cortical adrenal necrosis in, 899
universal serologic reaction with serum of, 414
Malignant pheochromocytoma and hypertension, 243
Maxted's filtrate, for extracting carbohydrates from streptococci, 880
Medicolegal examination of bodies recovered from burned buildings, 599
Mercuric chloride poisoning, serum lipids in, 962
Metal shadowing, optical microscopy of tissues, 63
Microdetermination of cholesterol, apparatus, 1076
Microslide, for culture of fungi, 278
Microtechnic
dioxane in dehydration, 291
frozen section, 873
instrument to level tissue, 897
metal shadowing, 63
paraffin tissue-block trimmer, 1070
tissue dehydration and embedding, 1061
use of sponge in biopsy, 92
use of sponge to obtain gastric tissue, 891
Microtome knife sharpener, 1089
Mima polymorpha, 1143
Mucopolysaccharide content in skin in myxedema, 442
Multiple blood specimens, collection of, 293
Multiple sclerosis, interpretation of cerebrospinal findings in, 16
Mycobacterium tuberculosis
from sputum or gastric washings, 91
streptomycin sensitivity of, 67, 76
Myoblastoma, granular cell, neurogenesis of, 522
Myometrial arteries, foam cell, intimal plaques, 30
Myxedema, pretibial, mucopolysaccharide content in skin, 442
Needle aspiration in diagnosis of lung carcinoma, 235
Nephrectomy, serum lipids following, 962
Neurinoma of small intestine, 827
Neurogenesis of granular cell myoblastoma, 522
Neuropathology, 1154
New methylene, a reticulocyte stain, 895
News and notices
Alabama Association of Pathologists, 859
American Association of Blood Banks, 859
American Board of Pathology, Diplomates, 400
American Society of Medical Technologists, 500
Canadian Society of Laboratory Technologists, 300
Chicago Medical School, 400
Dr. Kano Ikeda, an appreciation to, 300
letter of correction, 658
letters to the editor, 399, 598
Michigan Pathological Society, 400
Minnesota Society of Clinical Pathologists, 860
official jewelry, 98
Pennsylvania Association of Clinical Pathologists, 859
personal, 859
refresher courses in laboratory diagnosis, 860
thick film diagnosis of malaria, 859
Twenty-Seventh Annual Meeting, American Society of Clinical Pathologists, 52
Western Missouri Society of Pathologists, 860
Nitrogen mustard in Hodgkin's disease, 372
Nomograph, for calculation of icterus index, 884

Normal persons, universal serologic reaction with serum of, 347
Not for Ourselves Alone: Presidential Address, 999
Obituaries
Boerner, Frederick, 597
Couret, John Stephen, 199
Dyke, Louis H., Jr., 199
Edgar, James Day, 199
Favorite, Grant Orante, 200
Harris, Albert M., 199
Jones, Walter Clinton, 1166
Kelly, William Edward, 1167
Koritschoner, Robert, 1165
Larimore, Louise Doddridge, 597
Maldeis, Howard J., 1165
Pugliese, Frank Anthony, 1167
Rhamy, Bonnelle William, 1163
Schade, August Henry, 200
Spitz, Herman, 1166
Thompson, Harold A., 1166
Wurtz, John George, 200
Optical microscopy of tissues by metal shadowing, 63
Ovarian experimental tumors in rats, 501
Pancreatic acini, dilatation of, in hyperemesis of pregnancy, 846
Papanicolaou stain for amebae, 81
Para-aminosalicylic acid blood levels, 886
Paraffin tissue-block trimmer, 1070
Parasitology
Papanicolaou stain for amebae, 81
protozoan cysts, staining of, 84
Pathologic anatomy
actinomycosis, 201
amyloid, atypical, solitary "tumors", 1097
bone formation in heart, 974
bronchial adenoma, 836
cancer of cervix, progression from "precancer" stage, 770
clinicopathologic conferences, 177, 388, 576, 643, 789, 1140
cytologic diagnosis of cancer
bronchial secretions, 320
bronchiogenic carcinoma, 765
carcinoma of prostate, 315
from material aspirated from stomach, 328
pleural and peritoneal fluids, 309
symposium, 301
uterine cancer, 301
diffusely enlarged uterus, 544
epidermoid cyst of spleen, 558
esophagus varices, occurrence in routine autopsies, 554
experimental
cortical necrosis of adrenal, 899
nitrogen mustard in Hodgkin's disease, 372
ovarian tumors in rats, 501
studies in phagocytosis, 163
tumor cells in bronchial secretions, 320
foam cell intimal plaques in myometrial arteries, 30
Gaucher's disease in a Negro, 570
granulomas in bone marrow in herpes zoster, 981
Hashimoto's and Riedel's strumas and lymphadenoid goiter, 750
hyperemesis of pregnancy, pancreatic acini in, 846
interstitial cell tumor of testis, 1039
intra-uterine anoxia, changes in liver from, 801
leiomyoma angiomatosum of the mesentery, 639
leiomyosarcoma of stomach, 966
lung carcinoma, needle aspiration, 235
malignant pheochromocytoma, 243
neurinoma of small intestine, 827
neurogenesis of granular cell myoblastoma, 522
obstructive laryngeal papilloma, simulated homocide, 565
paracolon endocarditis, 840
sarcoidosis, clinicopathologic review of 300 cases, 725
Schistosoma japonicum infection and rectal carcinoma, 985
Pathologist, the, and continuation of medical education, 369
Pathology, widening horizons in, 1
Penicillin-resistant staphylococci, 1083
Phagocytosis, studies on, 163
Phenosulfonphthalein excretion, effect of caronamide on, 381
Phosphatases, value in blood, serum cytolysates and erythrocytic suspensions, 167
Photographic recording of hemolysis, 676
Pipetting instrument for serologic use, 693
Plasma, fresh and stored, with antithrombin activity, 1024
Plasmas, prothrombin-free, 1071
Platelet counts in rabbits, 1113, 1122
Platelets, Texchrome staining of, 681
Poliomyelitis, virus of, 1135

Potassium, in plasma and urine, by flame photometry, 461
Pregnancy tests, *Xenopus laevis* in, 284
Presidential addresses, 1, 999
Primary isolation of dermatophytes, 95
Primary splenic neutropenia, 48
Prism mirror, 96
Protein in edema fluid, 488
Prothrombin
 consumption time, coagulation mechanism in, 1016
 determination of, 471
 recent advances in our knowledge of, 41
 values, interpretation of, 1016
Protozoan cysts, iron-hematoxylin staining, 84
Rabbit lung, thromboplastin from, 71
Rats, experimental ovarian tumors in, 501
Red cells, agglutinability, in pyogenic infections, 782
Replacement transfusion, in treatment of reaction from incompatible blood, 1131
Reticulocyte counts, use of Köhler illumination, 883
Reticulocyte stain, new methylene blue, 895
Rh antibody titrations, survey of accuracy of, 1032
Rh-Hr antibodies in maternal serum, 80
Rh^{w}, further studies in, 779
Round cell infiltration, 393
Salmonellas, identification of, 106
Sarcoidosis, clinicopathologic review of 300 cases, 725
Schistosoma japonicum infection and rectal carcinoma, 985
Sedimentation tubes, support for, 298
Septicemia, fulminating, caused by *Mima polymorpha*, 1143
Serologic reaction with lipid antigen
 in lepromatous leprosy, 408
 in malaria, 414
 in syphilis, 401
Serology
 abnormal agglutinability of red cells in pyogenic infection, 782
 antibodies of third order, further evidence for, 134
 anti-Rh agglutinins, 435
 cardiolipin antigen
 in Kolmer test, 25
 specificity of, 156
 cerebrospinal findings in multiple sclerosis, 16
 cold agglutinins associated with acute hemolytic anemia, 630
 collecting multiple blood specimens, 293
 colloidal gold reagent, 86
 complement-fixation
 cardiolipin antigen in Kolmer test, 25
 pooled sheep cells in hemolytic system, 152
 quantitative measurement, 141
 group-specific substances, use in group O transfusions, 536
 infectious mononucleosis, macroscopic test, 665
 Kline test for syphilis, 274
 pipetting instrument, 693
 Rh-Hr antibodies, 80
 sheep blood, collection and dispensing, 496
 silicone-coated needles, 695
 silicone-treated test tubes, 296
 studies on blood factor rh^{w}, 621, 779
 survey of accuracy of Rh antibody titrations, 1032
 titration of antibodies, 265
 universal serologic reaction with lipid antigen
 in animals, 361
 in lepromatous leprosy, 408
 in malaria, 414
 in normal persons, 347
 in syphilis, 401
 in tuberculosis, 419
Serum lipids, in mercuric chloride poisoning, following nephrectomy, 962
Serum sodium, 659
Serum total base, 659
Sheep blood, collection and dispensing, apparatus for, 496
Sheep cells
 pooled, in the complement-fixation test, 152
 preservation of, 665
Silicone-coated needles, 695
Silicone-treated test tubes, 296
Silver, excretion of, in argyrosis, 668
Skin, localized myxedema, 442
Slide-culture technic, streptomycin sensitivity of *M. tuberculosis*, 76
Sodium and potassium in plasma and urine, 461
Solid-and-liquid medium for blood culture, 697
Splenic dyscrasias, lecithinase activity in, 615

Splenic extracts, effect on blood platelet counts in rabbits, 1122
Splenic neutropenia, primary, 48
Sponge
 gastric for tissue, for biopsy, 891
 use of, for biopsy, 92
Sputum, recovery of *M. tuberculosis* from, 91
Staining, iron hematoxylin for protozoan cysts, 84
Stapyhlococci, penicillin-resistant, 1083
Sternal marrow studies, in Hodgkin's disease, 929
Stilbamidine therapy, massive, of multiple myeloma, 634
Streptomycin sensitivity of *Mycobacterium tuberculosis*
 Dubos' medium, 67
 slide-culture technic, 76
Supplement, The American Board of Pathology, February
Survey of operation of blood banks, 35
Suspension medium for erythrocyte counts, 88
Syphilis
 specificity of cardiolipin antigens in tests for, 156
 universal serologic reaction with serum of, 401
Testis, interstitial cell tumor of, 1039
Texchrome silhouette staining of platelets, 681
Therapeutic fractions of human blood serum, production of, 10
Thin blood film, preparation, 98
Thromboembolism, blood platelets adhesiveness in, 701
Thromboplastin, from rabbit lung, 71
Tissue dehydrating, apparatus, 1061
Tissue embedding, apparatus, 1061
Titrations of Rh antibody, survey of accuracy of, 1032
Transfusions of incompatible blood, 1131
Trichrome stain, 898
Trisodium phosphate, for acid-fast bacilli in sputum, 688
Tubercle bacilli, culture of, from gastric juice, 685
Tuberculosis, universal serologic reaction with serum of, 419
Tumor cells in bronchial secretions, 320
Two-stage method for prothrombin, 471
Ultrafiltration, effect on antithrombin activity of plasma, 1024
Universal serologic reaction with lipid antigen
 in animals, 361
 in lepromatous leprosy, 408
 in malaria, 414
 in normal persons, 347
 in syphilis, 401
 in tuberculosis, 419
Urinary sugar test for blind, 500
Uterine cancer, cytologic diagnosis by examination of vaginal and uterine secretions, 301
Uterus, diffusely enlarged, 544
Vaccination, apparatus, 492
Varices in esophagus, 554
Ward Burdick Award Lecture, The Dynamic Equilibrium, 99
Water connections, 499
Xenopus laevis, in pregnancy tests, 284

INDEX OF AUTHORS

Abrahamson, E. M., 1076
Alton, O. M., and Platt, W. R., 536
Altschul, R., and Friesen, A. M., 163
Appel, M., and Bronk, T. T., 320
Arata, J. E.: See Rabson, S. M., 750
Armstrong, B. W.: See Wilson, B. J., 886
Atlas, D. H., Cash, M. M., and Kirschen, M. M., 962
Aull, J. C., Switzer, P. K., Ownby, F. D., and Milan, J. W., 488
Awny, A. J.: See Scheff, G. J., 615
Axelrod, E. C.: See Berman, L., *et al.*, 127
Ayre, J. E., and Ayre, W. B., 770
Ayre, W. B.: See Ayre, J. E., 770
Baggenstoss, A. H.: See Weed, L. A., 201
Baribeau, B. J.: See Kahn, R. L., 361
Baribeau, B. J.: See Kahn, R. L., *et al.*, 408
Barr, L. M.: See Ecker, E. E., *et al.*, 259
Bass, A. C.: See Overman, R. R., *et al.*, 907
Batchelor, T. M.: See Mosher, R. E., *et al.*, 461
Bauer, R. D.: See Erf, L. A., 372
Bauer, W. H., and Kuzma, J. F., 1097
Behrendt, H., 167
Bennett, G. A., and Paul, J. R., 177
Berman, L., Axelrod, A. R., Vonder Heide, E. C., and Sharp, E. A., 127
Binford, C. H.: See Gledhill, E. Y. *et al.*, 235
Binford, C. H., and Spriggs, J. B., 388
Bird, E. J.: See Mosher, R. E., *et al.*, 461
Birkhaug, K., 492
Bishop, F. H.: See Richardson, H. L., *et al.*, 328
Biskind, G. R., and Biskind, M. S., 501
Biskind, M. S.: See Biskind, G. R., 501
Boger, W. P., and Crosson, J. W., 381
Bohrod, M. G., 344
Bossak, H. N., 496
Boyd, W., 341
Boyle, A. J.: Mosher, R. E., *et al.*, 461
Brecher, G., 895
Brines, O. A., 653, 999, 1155
Bronk, T. T.: See Appel, M., 320
Burns, E.: See McGraw, J. J., *et al.*, 1004
Byrd, C. L.: See Young, V. M., *et al.*, 1135
Cannon, P. R., 99
Cary, S. G.: See Longfellow, D., *et al.*, 880
Cash, M. M.: See Atlas, D. H., *et al.*, 962
Chamovitz, J., and Fanger, H., 243
Chapman, S.: See Hertzog, A. J., *et al.*, 1139
Choisser, R. M., and Montgomery, R. R., 570
Cholak, J.: See Dutra, F. R., *et al.*, 229
Clark, M.: See Ricker, W., 725
Cleary, E.: See Turner, R. D., *et al.*, 296
Conger, M. E., 1070
Conklin, W. S.: See Richardson, H. L., *et al.*, 323
Conley, J. E., Erbes, J., Stoddard, F. J., and Frye, J. W., 1131
Conley, R. H., and Olwin, J. H., 966
Corper, H. J., and Nelson, C. R., 269
Cottrell, J. E.: See McDearman, S. C., 156
Covey, E. P., 500
Crosson, J. W.: See Boger, W. P., 381
Curphey, T. J., 1
Custer, R. P.: See Fust, J. A., 522
Davis, A. K.: See Overman, R. R., *et al.*, 907
Dittebrandt, M., 284
Dutra, F. R., 599
Dutra, F. R., Cholak, J., and Hubbard, D. M., 229
Ecker, E. E., and Hiatt, C. W., 141
Ecker, E. E., Hiatt, C. W., and Barr, L. M., 259
Edelberg, R.: See Love, W. E., *et al.*, 676
Ellias, E. P.: See Linn, H. J., 558
Elton, N. W.: See Giammalvo, J. T., *et al.*, 76
Engleson, G., and Grubb, R., 782
Erbes, J.: See Conley, J. E., *et al.*, 1131
Erf, L. A., and Bauer, R. D., 372
Erf, L. A., and Fry, K. E., 48
Fanger, H.: See Chamovitz, J., 243
Faust, J. and Hood, M., 1143
Felsen, J., 289
Felsenfeld, O., 106
Felsenfeld, O.: See Young, V. M., *et al.*, 1135
Finestone, A. J., and Geschickter, C. F., 974
Fink, H.: See Meyer, L. M., *et al.*, 119
Fredenburgh, E. J., 86
Freeman, W., 345
French, E. M.: See Turner, R. D., *et al.*, 296
Frey, A., and Frey, M., 699

Frey, M.: See Frey, A., 699
Friedman, I. A., and Goldin, M., 840
Friesen, A. M.: See Altschul, R., 163
Frisch, A. W., and Jackets, V., 435
Frost, R. J.: See Giordano, R. J., *et al.*, 25
Fry, K. E.: See Erf, L. A., 48
Frye, J. W.: See Conley, J. E., *et al.*, 1131
Fust, J. A., and Custer, R. P., 522
Geever, E. F., Williams, W. S., and McWilliams, J. E., 836
Gerbasi, F. S., and Robinson, A. R., 668
Geschickter, C. F.: See Finestone, A. J., 974
Giammalvo, J. T., Natsios, G. A., and Elton, N. W., 76
Giordano, A. S., Frost, R. J., and Higginbotham, M. W., 25
Gladstone, S. A., 92, 891
Gledhill, E. Y., Spriggs, J. B., and Binford, C. H., 235
Golden, A., 918
Golden, A., and Overman, R. R., 899
Golden, A.: See Overman, R. R., *et al.*, 907
Goldin, M.: See Friedman, I. A., 840
Gollub, S., Kaplan, F. E., Meranze, D. R., and Tuft, H., 1071
Gordon, E. B.: See Wiener, A. S., 621
Greenspan, E. M.: See Haedicke, T. A., 634
Greenwalt, T. J., 695
Grubb, R.: See Engleson, G., 782
Gruenwald, P., 801
Gurevitch, J., Polishuk, Z., and Hermoni, D., 265
Guy, R.: See Hill, J. M., *et al.*, 134
Haberman, S.: See Hill, J. M., *et al.*, 134
Haedicke, T. A., and Greenspan, E. M., 634
Halbert, B., and Stone, S. N., 576
Harris, A. H.: See Lange, C., 16
Hartz, Ph. H., 846
Hartz, Ph. H., and van de Stadt, F. R., 639
Hazard, J. B., and Stevenson, G. F., 873
Herbut, P. A., 315, 343
Hermoni, D.: See Gurevitch, J., *et al.*, 265
Herring, J.: See Hertzog, A. J., *et al.*, 1139
Hertzog, A. J., Chapman, S., and Herring, J., 1139
Hiatt, C. W.: See Ecker, E. E., 141
Hiatt, C. W.: See Ecker, E. E., *et al.*, 259
Higginbotham, M. W.: See Giordano, R. J., *et al.*, 25
Hill, J. H., 985
Hill, J. M., Haberman, S., and Guy, R., 134
Hood, M.: See Faust, J., 1143
Horn, R. C.: See Reiners, C. R., 1039
Hosley, R. J., 884
Hubbard, D. M.: See Dutra, F. R., *et al.*, 229
Hunter, C. A., and Victor, F., 293
Hunter, W. C.: See Richardson, H. L., *et al.*, 323
Iseri, L. T.: See Mosher, R. E., *et al.*, 461
Jackets, V.: See Frisch, A. W., 435
Jacobson, A. S.: See Rubin, I. L., *et al.*, 630
Jacobson, S. D.: See Mosher, R. E., *et al.*, 461
Jernstrom, P., and Perkins, E., 700
Johnson, D. A., 84
Jolly, P. N.: See McGuire, J., 643
Jones, F.: See Reid, A. F., 10
Kabler, P., and Lundholm, R., 685
Kahn, R. L., 347, 414, 419
Kahn, R L., and Baribeau, B. J., 361
Kahn, R. L., Baribeau, B. J., and Villalon, F. T., 408
Kahn, R. L., and McDermott, E. B., 401
Kaplan, F. E.: See Gollub, S., *et al.*, 1071
Kaump, D. H.: See Truemner, K. M., 544
Kennedy, J. D., 883
Kernohan, J. W., 1154
Kirschbaum, J. D., 789
Kirschen, M. M.: See Atlas, D. H., *et al.*, 962
Kline, B. S., and Suessenguth, H., 274
Kuhn, L. R.: See Longfellow, D., *et al.*, 880
Kuzma, J. F.: See Bauer, W. H., 1097
Landau, R. L., 424
Lange, C., and Harris, A. H., 16
Laskaris, T., 95
Lawless, M. C.: See Turner, R. D., *et al.*, 296
Lewis, W. G.: See Tarshis, M. S., 688
Limarzi, L. R., and Paul, J. T., 929
Lind, H. E., 72
Linn, H. J., and Ellias, E. P., 588
Lippman, R. W., 499
Littman, M. L., 278
Littman, M. L., McQuown, A. L., and Schneidau, J. D., 865
Lockard, C. D., 91
Lonergan, A. M.: See Rice, W. G., 1083
Longfellow, D., Cary, S. G., and Kuhn, L. R., 880
Love, W. E., Edelberg, R., and MacNichol, E. F., 676
Lundholm, R.: See Kabler, P., 685

MacNichol, E. F.: See Love, W. E., *et al.*, 676
Mann, F. D., 861
Markell, E. K.: See Michael, P., 81
Marsters, R. W., 1032
Marvin, H. N.: See Wells, B. B., *et al.*, 448
Marwin, R. M., 697
Mazzarino, C. A.: See Wiener, A. S., *et al.*, 779
McDearman, S. C., and Cottrell, J. E., 156
McDermott, E. B.: See Kahn, R. L., 401
McDonald, J. R.: See Woolner, L. B., 765
McGraw, J. J., Strumia, M. M., and Burns, E., 1004
McGuire, J., and Jolly, P. N., 643
McQuown, A. L.: See Littman, M. L., *et al.*, 865
McWilliams, J. E.: See Geever, E. F., *et al.*, 836
Meranze, D. R.: See Gollub, S., *et al.*, 1071
Meyer, L. M.: See Rubin, I. L., *et al.*, 630
Meyer, L. M., Fink, H., Sawitsky, A., Rowen, M., and Ritz, N. D., 119
Michael, P., and Markell, E. K., 81
Milan, J. W.: See Aull, J. C., *et al.*, 488
Milligan, M., 897
Mollov, M., and Oshinsky, A., 67
Montgomery, R. R.: See Choisser, R. M., 570
Moolten, S. E., and Vroman, L., 701
Moolten, S. E., Vroman, L., and Vroman, G. M. S., 814
Mosher, R. E., Boyle, A. J., Bird, E. J., Jacobson, S. D., Batchelor, T. M., Iseri, L. T., and Myers, G. B., 461
Muraschi, T. F., and Tompkins, V. N., 152
Myers, G. B.: See Mosher, R. E., *et al.*, 461
Natsios, G. A.: See Giammalvo, J. T., *et al.*, 76
Nelson, C. R.: See Corper, H. J., 269
Nichols, J., 96
Norris, R. F., 35
Olwin, J. H., See Conley, R. H., 966
Oshinsky, A.: See Mollov, M., 67
Overman, R. R.: See Golden, A., 899
Overman, R. R., Bass, A. C., Davis, A. K., and Golden, A., 907
Ownby, F. D.: See Aull, J. C., *et al.*, 488
Papanicolaou, G. N., 301, 346
Paul, J. R.: See Bennett, G. A., 177
Paul, J. T.: See Limarzi, L. R., 929
Pearce, R. H.: See Watson, E. M., 442
Perkins, E.: See Jernstrom, P., 700
Petersen, A. B.: See Richardson, H. L., *et al.*, 323
Platt, W. R.: See Alton, O. M., 536
Polishuk, Z: See Gurevitch, J., *et al.*, 265
Popper, H., Steigmann, F., and Szanto, P. B., 710
Popper, H., and Volini, I. F., 1146
Queen, F. B.: See Richardson, H. L., *et al.*, 328
Quick, A. J., 1016
Rabson, S. M., 346, 393
Rabson, S. M., and Arata, J. E., 750
Ralph, P. H.: See Scheff, G. F., 1113
Rappaport, F., and Skariton, M., 88, 665
Rebuck, J. W., 217
Reid, A. F., and Jones, F., 10
Reiners, C. R., and Horn, R. C., 1039
Rice, W. G., 1089
Rice, W. G., and Lonergan, A. M., 1083
Richardson, H. L., Hunter, W. C., Conklin, W. S., and Petersen, A. B., 323
Richardson, H. L., Queen, F. B., and Bishop, F. H., 328
Ricker, W., and Clark, M., 725
Ritz, N. D.: See Meyer, L. M., *et al.*, 119
Robinson, A. R.: See Gerbasi, F. S., 668
Rosahn, P. D., 796
Rosbash, D. O., 297
Rowen, M.: See Meyer, L. M., *et al.*, 119
Rubin, I. L., Jacobson, A. S., and Meyer, L. M., 630
Sano, M., 898
Saphir, O., 309
Sawitsky, A.: See Meyer, L. M., *et al.*, 119
Scheff, G. J., and Awny, A. J., 615
Scheff, G. J., and Ralph, P. H., 1113
Scheff, G. J., and Scheff, I. M., 1122
Scheff, I. M.: See Scheff, G. J., 1122
Scher, Y., 71
Schleicher, W. M., 981
Schneidau, J. D.: See Littman, M. L., *et al.*, 865
Scott, D. B., and Wyckoff, R. W. G., 63
Seegers, W. H., and Ware, A. G., 41
Seegers, W. H.: See Ware, A. G., 471
Sharp, E. A.: See Berman, L., *et al.*, 127
Sheehan, J. F., 30
Skariton, M.: See Rappaport, F., 88, 665
Slattery, M., 80
Spelman, J. W., 565
Spriggs, J. B.: See Binford, C. H., 388
Spriggs, J. B.: See Gledhill, E. Y., *et al.*, 235
Stefanini, M., 1024
Steigmann, F.: See Popper, H., *et al.*, 710
Stevenson, G. F.: See Hazard, J. B., 873

Stewart, H. L., 1095
Stoddard, F. J.: See Conley, J. E., *et al.*, 1131
Stone, S. N.: See Halbert, B., 576
Strumia, M. M.: See McGraw, J. J., *et al.*, 1004
Strumia, M. M., Wall, R., Strumia, P. V., 483
Strumia, P. V.: See Strumia, M. M., 483
Suessenguth, H.: See Kline, B. S., 274
Sunderman, F. W., 659
Svedmyr, A., 693
Switzer, P. K.: See Aull, J. C., *et al.*, 488
Szanto, P. B.: See Popper, H., *et al.*, 710
Tannenberg, J., 1061
Tarshis, M. S., and Lewis, W. G., 688
Tompkins, V. N.: See Muraschi, T. F., 152
Truemner, K. M., and Kaump, D. H., 544
Tuft, H.: See Gollub, S., *et al.*, 1071
Turner, R. D., Lawless, M. C., Cleary, E., and French, E. M., 296
Unger, L. J.: See Wiener, A. S., *et al.*, 779
van de Stadt, F. R.: See Hartz, Ph. H., 639
Victor, F.: See Hunter, C. A., 293
Villalon, F. T.: See Kahn, R. L., *et al.*, 408
Volini, I. F.: See Popper, H., 1146
Vonder Heide, E. C.: See Berman, L., *et al.*, 127
Vroman, G. M. S.: See Moolten, S. E., *et al.*, 814
Vroman, L., 681
Vroman, L.: See Moolten, S. E., 701
Vroman, L.: See Moolten, S. E., *et al.*, 814
Wachstein, M., 608
Waldvogel, M.: See Wells, B. B., *et al.*, 448
Walker, P. E., 827
Walker, T. F., 291
Wall, R.: See Strumia, M. M., *et al.*, 483
Ware, A. G.: See Seegers, W. H., 41
Ware, A. G., and Seegers, W. H., 491
Watson, E. M., and Pearce, R. H., 442
Weed, L. A., and Baggenstoss, A. H., 201
Weinberg, T., 554
Wells, A. H., 369
Wells, B. B., Marvin, H. N., and Waldvogel, M., 448
Wiener, A. S., and Gordon, E. B., 621
Wiener, A. S., Unger, L. J., and Mazzarino, C. A., 779
Williams, W. S.: See Geever, E. F., *et al.*, 836
Wilson, B. J., and Armstrong, B. W., 886
Woolner, L. B., and McDonald, J. R., 765
Wyckoff, R. W. G.: See Scott, D. B., 63
Young, V. M., Felsenfeld, O., and Byrd, C. L., 1135

American Journal of Clinical Pathology

S. E. Gould, M.D., *Editor*

Volume 19

Baltimore
The Williams & Wilkins Company
1949

TABLE OF CONTENTS

Number 1, January 1949

Widening Horizons in Pathology. T. J. Curphey 1
Production of Therapeutic Fractions of Human Blood Serum. Removal of Salts with Ion-Exchange Resins. A. F. Reid and F. Jones 10
Interpretation of Findings in Cerebrospinal Fluid. III. Syndrome of Multiple Sclerosis. C. Lange and A. H. Harris 16
Cardiolipin Antigen in the Kolmer Complement-Fixation Test. A. S. Giordano, R. J. Frost and M. W. Higginbotham 25
Foam Cell Plaques in Intima of Myometrial Arteries. A Presumptive Sign of Previous Irradiation. J. F. Sheehan 30
A Survey of the Direction and Operation of the Blood Banks of Ten Large Hospitals. R. F. Norris 35
Recent Advances in Our Knowledge of Prothrombin. A Brief Review. W. H. Seegers and A. G. Ware 41
Case Report: Primary Splenic Neutropenia. L. A. Erf and K. E. Fry 48
Editorial: Dr. Giordano, Our True Physician 51
News and Notices 52

Technical Section

Metal Shadowing for the Optical Microscopy of Certain Tissues. D. B. Scott and R. W. G. Wyckoff 63
Value of Dubos' Medium in Tests for Streptomycin Sensitivity of *Mycobacterium tuberculosis*. M. Mollov and A. Oshinsky 67
Preparation of Thromboplastin from Rabbit Lung. Y. Scher 71
Limitations of Flourescent Microscopy for Detection of Acid-Fast Bacilli. H. E. Lind. 72
Streptomycin Sensitivity of *Mycobacterium tuberculosis*. A Slide-Culture Technic. J. T. Giammalvo, G. A. Natsios and N. W. Elton 76
Testing for Rh-Hr Antibodies in Maternal Serum. A Satisfactory Source of Erythrocytes. M. Slattery 80
Modified Papanicolaou Stain for Amebae. P. Michael and E. K. Markell 81
Concentration of Protozoan Cysts for Iron Hematoxylin Staining. D. A. Johnson 84
Preparation of Colloidal Gold Reagent. E. J. Fredenburgh 86
An Improved Suspension Medium for Estimation of Erythrocyte Counts. F. Rappaport and M. Skariton 88
Recovery of *Mycobacterium tuberculosis* from Sputum or Gastric Washings. Use of 250 ml. Centrifuge Bottle. C. D. Lockard 91
Use of Sponge in the Collection and Preparation of Tissue for Biopsy. S. A. Gladstone. 92
A Simple Efficient Method for Primary Isolation of Dermatophytes. T. Laskaris 95
An Economical Prism Mirror. J. Nichols 96
Technical Suggestions 98
Notice 98

Number 2, February 1949

The Dynamic Equilibrium. P. R. Cannon 99
An Outline for the Identification of Salmonellas. O. Felsenfeld 106
Aminopterin (A Folic Acid Antagonist) in the Treatment of Leukemia. L. M. Meyer, H. Fink, A. Sawitsky, M. Rowen and N. D. Ritz 119
Use of a Folic Acid Antagonist in Chronic Leukemia. L. Berman, A. R. Axelrod, E. C. Vonder Heide and E. A. Sharp 127
Further Evidence for Antibodies of Third Order. Fractionation of Agglutinins, Block-

ing Antibodies and Cryptagglutinoids by Physicochemical Methods. J. M. Hill, S. Haberman and R. Guy 134
A Method for the Quantitative Measurement of Complement-Fixation. E. E. Ecker and C. W. Hiatt 141
Pooled Sheep Cells for Use in the Hemolytic System of the Complement-Fixation Test. T. F. Muraschi and V. N. Tompkins 152
Specificity of Cardiolipin Antigens in Tests for Syphilis. S. C. McDearman and J. E. Cottrell 156
Studies on Phagocytosis. II. R. Altschul and A. M. Friesen 163
Phosphatases in Blood of Man. Values in Whole Blood, Plasma, Cytolysates and Erythrocytic Suspensions. H. Behrendt 167
Clinicopathologic Conference. G. A. Bennett and J. R. Paul 177
Selected Abstracts 183
Book Reviews 192
Obituaries 199
Supplement. The American Board of Pathology. List of Certified Pathologists.

NUMBER 3, MARCH 1949

Actinomycosis. A Pathologic and Bacteriologic Study of Twenty-One Fatal Cases. L. A. Weed and A. H. Baggenstoss 201
A Simple Direct Method for the Electron Microscopy of Peripheral Blood Cells. J. W. Rebuck 217
The Value of Beryllium Determinations in the Diagnosis of Berylliosis. F. R. Dutra, J. Cholak and D. M. Hubbard 229
Needle Aspiration in the Diagnosis of Lung Carcinoma. Report of Experience with Seventy-Five Aspirations. E. Y. Gledhill, J. B. Spriggs and C. H. Binford 235
Case Report: Malignant Pheochromocytoma and Hypertension. J. Chamovitz and H. Fanger 243
Selected Abstracts 252
Book Reviews 256
Books Received 258

Technical Section

A Method of Determining the Distribution of Erythrocyte Fragility in Human Blood. E. E. Ecker, C. W. Hiatt and L. M. Barr 259
Titration of Physiologic and Immune Antibodies with Washed and Unwashed Erythrocytes. J. Gurevitch, Z. Polishuk and D. Hermoni 265
Methods of Concentrating Acid-Fast Bacilli. H. J. Corper and C. R. Nelson 269
Standard Kline Test of Cerebrospinal Fluid for Syphilis. Employing Cardiolipin-Lecithin Antigen. B. S. Kline and H. Suessenguth 274
A Special Microslide for Culture of Fungi. M. L. Littman 278
Use of *Xenopus laevis* in Testing for Pregnancy. Analysis of 1100 Tests. M. Dittebrandt 284
The Divided Culture Plate. J. Felson 289
Dioxane Method of Dehydration of Tissues for Paraffin Embedding. T. F. Walker 291
An Improved Simplified Technic for Collecting Multiple Blood Specimens. C. A Hunter and F. Victor 293
Use of Silicone-Treated Test Tubes to Obtain Serum from Blood. R. D. Turner, M. C. Lawless, E. Cleary and E. M. French 296
Preparation of Folin-Ciocalteu's Phenol Reagent. Using 30 Per cent Hydrogen Peroxide. D. O. Rosbash 297
A Convenient Support for Sedimentation Tubes. E. M. Abrahamson 298
News and Notices 300

NUMBER 4, APRIL 1949

Symposium on the Cytologic Diagnosis of Cancer
Cytologic Diagnosis of Uterine Cancer by Examination of Vaginal and Uterine Secretions. G. N. Papanicolaou ... 301
Cytologic Diagnosis of Cancer from Pleural and Peritoneal Fluids. O. Saphir ... 309
Cytologic Diagnosis of Carcinoma of the Prostate. P. A. Herbut ... 315
Tumor Cells in Bronchial Secretions. An Experimental and Statistical Study. M. Appel and T. T. Bronk ... 320
A Cytohistologic Study of Bronchial Secretions. H. L. Richardson, W. C. Hunter, W. S. Conklin and A. B. Petersen ... 323
Cytohistologic Diagnosis of Material Aspirated from Stomach. Accuracy of Diagnosis of Cancer, Ulcer and Gastritis from Paraffin-Embedded Washings. H. L. Richardson, F. B. Queen and F. H. Bishop ... 328
Discussion of Papers. W. Boyd ... 341
General Discussion. P. A. Herbut, M. G. Bohrod, W. Freeman, S. M. Rabson and G. N. Papanicolaou ... 343
Universal Serologic Reaction with Lipid Antigen
I. In Normal Persons. R. L. Kahn ... 347
II. In Animals. R. L. Kahn and B. J. Baribeau ... 361
The Pathologist and Continuation of Medical Education in a Private Hospital. A. H. Wells ... 369
The Clinical Effect of Nitrogen Mustard on Hodgkin's Disease. L. A. Erf and R. D. Bauer ... 372
Effect of Caronamide on Excretion of Phenosulfonphthalein. W. P. Boger and J. W. Crosson ... 381
Clinicopathologic Conference. C. H. Binford and J. B. Spriggs ... 388
Editorial: "Round Cell Infiltration." S. M. Rabson ... 393
Selected Abstracts ... 394
Book Reviews ... 395
News and Notices ... 399

NUMBER 5, MAY 1949

Universal Serologic Reaction with Lipid Antigen.
III. In Syphilis. R. L. Kahn and E. B. McDermott ... 101
IV. In Lepromatous Leprosy. R. L. Kahn, B. J. Baribeau and F. T. Villalon ... 408
V. In Malaria. R. L. Kahn ... 414
VI. In Tuberculosis. R. L. Kahn ... 419
Diagnostic Significance and Laboratory Methods in Determination of the 17-Ketosteroids. R. L. Landau ... 424
Anti-Rh Agglutinins. Their Clinical Significance. A. W. Frisch and V. Jackets ... 435
The Mucopolysaccharide Content of the Skin in Localized (Pretibial) Myxedema. H. E. M. Watson and R. H. Pearce ... 442
Determination of Anti-Tryptic Activity in Human Plasma. B. B. Wells, H. N. Marvin and M. Waldvogel ... 448
Book Reviews ... 454
Selected Abstracts ... 460

Technical Section

The Use of Flame Photometry for the Quantitative Determination of Sodium and Potassium in Plasma and Urine. R. E. Mosher, A. J. Boyle, E. J. Bird, S. D. Jacobson, T. M. Batchelor, L. T. Iseri and G. B. Myers ... 461
Two-Stage Procedure for the Quantitative Determination of Prothrombin Concentration. A. G. Ware and W. H. Seegers ... 471

A Method for Estimation of Blood Volume. M. M. Strumia, R. Wall and P. V. Strumia 483
Rapid Method for Determining Edema Fluid Proteins. J. C. Aull, P. K. Switzer, F. D. Ownby and J. W. Milan 488
A Detachable Spring-Actuated Multiple Puncture Apparatus for BCG Vaccination. K. Birkhaug 492
An Apparatus for the Aseptic Collection and Dispensing of Sheep Blood. H. N. Bossak 496
Rapid Interchange of Laboratory Water Connections. R. W. Lippman 499
A Urinary Sugar Test for the Blind. E. P. Covey 500
Notice 500

NUMBER 6, JUNE 1949

Experimental Ovarian Tumors in Rats. G. R. Biskind and M. S. Biskind 501
On the Neurogenesis of So-Called Granular Cell Myoblastoma. J. A. Fust and R. P. Custer 522
An Evaluation of the Use of Group-Specific Substances in Group O Blood Transfusions. O. M. Alton and W. R. Platt 536
The Diffusely Enlarged Uterus. K. M. Truemner and D. H. Kaump 544
Observations on the Occurrence of Varices of the Esophagus in Routine Autopsy Material. T. Weinberg 554
Case Reports
Epidermoid Cyst of the Spleen. H. J. Linn and E. P. Ellias 558
Simulated Homicide. Obstructive Laryngeal Papilloma in a Child. J. W. Spelman 565
Gaucher's Disease in a Negro. R. M. Choisser and R. R. Montgomery 570
Clinicopathologic Conference. B. Halpert and S. N. Stone 576
Selected Abstracts 583
Book Reviews 591
Obituaries 597
News and Notices 598

NUMBER 7, JULY 1949

Medicolegal Examination of Bodies Recovered from Burned Buildings. F. R. Dutra 599
Studies on Inclusion Bodies. I. Acid-Fastness of Nuclear Inclusion Bodies That Are Induced by Ingestion of Lead and Bismuth. M. Wachstein 608
Lecithinase Activity in Splenic Dyscrasias. G. J. Scheff and A. J. Awny 615
Studies on the Blood Factor rh^w. A. S. Wiener and E. B. Gordon 621
Case Reports
Acute Hemolytic Anemia Associated with Elevated Titer of Cold Agglutinins. I. L. Rubin, A. S. Jacobson and L. M. Meyer 630
Massive Stilbamidine Therapy of Multiple Myeloma. T. A. Haedicke and E. M. Greenspan 634
Leiomyoma Angiomatosum of the Mesentery. Ph. H. Hartz and F. R. van de Stadt 639
Clinicopathologic Conference. J. McGuire and P. N. Jolly 643
Editorial: The Training of Clinical Pathologists. O. A. Brines 653
Selected Abstracts 654
Book Reviews 655
News and Notices 658

Technical Section

Rapid Measurement of Serum Total Base and Estimation of Serum Sodium. An Improved Conductivity Assembly. F. W. Sunderman 659

Rapid Macroscopic Test for Infectious Mononucleosis. A Method for Preservation of Sheep Cells. F. Rappaport and M. Skariton 665
Excretion of Silver in a Patient with Argyrosis. Ineffectiveness of Treatment with BAL. F. S. Gerbasi and A. R. Robinson 668
Photographic Recording of Hemolysis Using a Portable Electrocardiograph. W. E. Love, R. Edelberg and E. F. MacNichol 676
Texchrome Silhouette Staining of Platelets. Morphology *in Vitro* Before and During Coagulation. L. Vroman 681
Culture of Tubercle Bacilli from Specimens of Gastric Juice. P. Kabler and R. Lundholm 685
Use of Clorox and Trisodium Phosphate in Demonstration of Acid-Fast Bacilli in Sputum. M. S. Tarshis and W. G. Lewis 688
An Accurate Pipetting Instrument for Serologic Use. A. Svedmyr 693
The Use of Silicone-Coated Needles in Blood Donor Sets. T. J. Greenwalt 695
An Improved Blood Culture Bottle Utilizing a Solid-and-Liquid Medium. R. M. Marwin 697
The Determination of Bromsulphalein in the Presence of Hemolysis, Turbidity and Bile. A. Frey and M. Frey 699
A Source of Error in Bromsulphalein Tests. P. Jernstrom and E. Perkins 700

NUMBER 8, AUGUST 1949

The Adhesiveness of Blood Platelets in Thromboembolism and Hemorrhagic Disorders. I. Measurement of Platelet Adhesiveness by the Glass-Wool Filter. S. E. Moolten and L. Vroman 701
Quantitative Correlation of Morphologic Liver Changes and Clinical Tests. H. Popper, F. Steigmann and P. B. Szanto 710
Sarcoidosis. A Clinicopathologic Review of Three Hundred Cases, Including Twenty-Two Autopsies. W. Ricker and M. Clark 725
Hashimoto's and Riedel's Strumas and Lymphadenoid Goiter. S. M. Rabson and J. E. Arata 750
Cytologic Diagnosis of Bronchogenic Carcinoma. L. B. Woolner and J. R. McDonald 765
Progression from "Precancer" Stage to Early Carcinoma of Cervix within One Year. Combined Cytologic and Histologic Study with Report of a Case. J. E. Ayre and W. B. Ayre 770
Further Studies on the rh^w Factor. A. S. Wiener, L. J. Unger and C. A. Mazzarino 779
Abnormal Agglutinability of Red Cells in Pyogenic Infections. Report of Two Cases. G. Engleson and R. Grubb 782
Clinicopathologic Conference. J. D. Kirshbaum 789
Editorial: Pathways in Pathology. P. D. Rosahn 796

NUMBER 9, SEPTEMBER 1949

Degenerative Changes in the Right Half of the Liver Resulting from Intra-Uterine Anoxia. P. Gruenwald 801
Adhesiveness of Blood Platelets in Thromboembolism and Hemorrhagic Disorders. II. Diagnostic and Prognostic Significance of Platelet Adhesiveness. S. E. Moolten, L. Vroman and G. M. S. Vroman 814
Case Reports
Neurinoma of the Small Intestine. Its Relative Frequency among Tumors of that Region. P. E. Walker 827
Bronchial Adenoma with Cancerous Transformation. E. F. Geever, W. S. Williams and J. E. McWilliams 836
Paracolon Endocarditis. I. A. Friedman and M. Goldin 840
Dilatation of the Acini of the Pancreas in Hyperemesis of Pregnancy. Ph. H. Hartz. 846

Book Reviews 850
Selected Abstracts 856
News and Notices 859

Technical Section

Co-Thromboplastin Assay. A Means of Study of Abnormalities of Blood Coagulation. F. B. Mann 861
Recent Advances in the Culture Diagnosis of Fungus Disease. M. L. Littman, A. L. McQuown and J. D. Schneidau 865
A Frozen Section Technic. J. B. Hazard and G. F. Stevenson 873
Use of Maxted's Streptomyces Filtrate for Rapidly Extracting Group Carbohydrates from Hemolytic Streptococci. D. Longfellow, S. G. Cary and L. R. Kuhn 880
Use of Köhler Illumination for Reticulocyte Counts. J. D. Kennedy 883
Nomograph for Rapid Calculation of Icterus Index When Determined by Colorimeter. R. J. Hosley 884
Determination of Para-Aminosalicylic Acid Blood Levels. B. J. Wilson and B. W. Armstrong 886
Use of Sponge in Obtaining Tissue from Stomach for Biopsy. S. A. Gladstone 891
New Methylene Blue as a Reticulocyte Stain. G. Brecher 895
An Instrument to Level Tissue in Paraffin Embedding. M. Milligan 897
Trichrome Stain for Tissue Section, Culture or Smear. M. Sano 898

NUMBER 10, OCTOBER 1949

I. Experimental Cortical Necrosis of Adrenal Gland in the Monkey in Diphtheria and in Malaria. A. Golden and R. R. Overman 899
II. The Effect of Lipo-adrenal Extract on Ionic Balance in Fatal Simian Malaria. R. R. Overman, A. C. Bass, A. K. Davis and A. Golden 907
III. Cortical Necrosis of the Adrenal Gland in Man. A. Golden 918
Sternal Marrow Studies in Hodgkin's Disease. L. R. Limarzi and J. T. Paul 929
Serum Lipids Following Nephrectomy and In Mercuric Chloride Poisoning. D. H. Atlas, M. M. Cash and M. M. Kirschen 962
Case Reports
Leiomyosarcoma of the Stomach. R. H. Conley and J. H. Olwin 966
Bone Formation in the Heart. A. J. Finestone and C. F. Geschickter 974
Reticulo-epithelioid Cell Granulomas in Bone Marrow in Herpes Zoster. E. M. Schleicher 981
Asymptomatic *Schistosoma japonicum* Infection Associated with Rectal Carcinoma. J. H. Hill 985
Editorial: Howard T. Karsner 990
Book Reviews 992
Books Received 998

NUMBER 11, NOVEMBER 1949

Presidential Address: Not for Ourselves Alone. O. A. Brines 999
The Incidence of Posttransfusion Serum Hepatitis. J. J. McGraw, M. M. Strumia and E. Burns 1004
The Coagulation Mechanism. With Specific Reference to the Interpretation of Prothrombin Time Values and a Consideration of the Prothrombin Consumption Time. A. J. Quick 1016
Effect of Ultrafiltration and Carbon Dioxide on Antithrombin Activity of Fresh and Stored Human Plasma. M. Stefanini 1024
A Survey of the Accuracy of Rh Antibody Titrations in Several Hospital Laboratories. R. W. Marsters 1032
Interstitial Cell Tumor of the Testis. C. R. Reiners, Jr. and R. C. Horn, Jr. 1039

Book Reviews 1048
Selected Abstracts 1052

Technical Section

Automatic Tissue Dehydrating and Embedding Apparatus. J. Tannenberg 1061
Paraffin Tissue-Block Trimmer. M. E. Conger 1070
Critical Adsorption Levels and Electrophoretic Analyses of "Prothrombin-Free" Plasmas. S. Gollub, F. E. Kaplan, D. R. Meranze and H. Tuft 1071
An Improved Apparatus for the Microdetermination of Cholesterol. E. M. Abrahamson 1076
Penicillin-Resistant Staphylococci. W. G. Rice and A. M. Lonergan 1083
An Improvised Microtome Knife Sharpener. W. G. Rice 1089
Index of Subjects 1091
Index of Authors 1093
Table of Contents iii

NUMBER 12, DECEMBER 1949

The Pathology of Chemical Carcinogenesis. H. L. Stewart 1095
Solitary "Tumors" of Atypical Amyloid (Paramyloid). W. H. Bauer and J. F. Kuzma 1097
An Improved Method of Counting Blood Platelets. G. J. Scheff and P. H. Ralph ...
Effect of Splenic Extracts on Blood Platelet Counts in Rabbits. G. J. Scheff and I. M. 1113
Scheff 1122
Replacement Transfusion Following Administration of Incompatible Blood. Report of Case with Successful Outcome. J. E. Conley, J. Erbes, F. J. Stoddard and J. W. Frye 1131
Behavior of Virus of Poliomyelitis in a Culture of *Endamoeba Histolytica*. V. M. Young, O. Felsenfeld and C. L. Byrd 1135
Congenital Pulmonary Aspiration-Tuberculosis. Report of a Case. A. J. Hertzog, S. Chapman and J. Herring 1139
Fulminating Septicemia Caused by *Mima polymorpha*. Report of Case. J. Faust and M. Hood 1143
Clinicopathologic Conference. H. Popper and I. F. Volini 1146
Editorials: Neuropathology. J. W. Kernohan 1154
What is the Trend? O. A. Brines 1155
On Shortening Scientific Papers 1155
Book Reviews 1156
Selected Abstracts 1161
Obituaries 1166
Index of Subjects 1169
Index of Authors 1183
Table of Contents iii

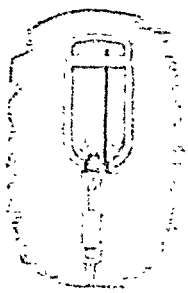


BAXTER

Blood Banking

WITHOUT WASTE

The Baxter method of collecting, storing and administering blood and plasma is a model of simplicity, safety and streamlined efficiency. The closed system, developed and introduced by Baxter, insures sterility. Baxter expendable donor and administration sets make procedures simple, safe, expedites teaching. And now the new Baxter Fuso-Flo stopper solves the aging problem, insuring trouble-free, easy-flowing infusions. A demonstration of this complete Baxter program can be arranged without obligation.



Manufactured by
BAXTER LABORATORIES, INC.
Morton Grove, Illinois

Produced and distributed in the eleven western states by DON BAXTER, Inc., Glendale, California

DISTRIBUTED AND AVAILABLE ONLY IN THE 37 STATES EAST OF THE ROCKIES (except in the city of El Paso, Texas) THROUGH

AMERICAN HOSPITAL SUPPLY CORPORATION

GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

Board of Registry Offers Limited Certification in Bacteriology, Chemistry and Histologic Technic

Special examinations in these fields are now given to candidates who meet the following requirements:

Histologic Technic

Graduation from a high school approved by the proper qualifying body; plus one year of training and experience in histologic technic.

Bacteriology

Bachelor's degree in Bacteriology from an institution accredited by a recognized standardizing body; plus one year of experience in bacteriology in an acceptable laboratory.

Chemistry

Bachelor's degree in Chemistry (including certain specified courses) from an institution accredited by a recognized standardizing body; plus one year of experience in chemistry in an acceptable medical laboratory.

REGISTRY OF MEDICAL TECHNOLOGISTS

of the

American Society of Clinical Pathologists

Muncie, Indiana

Advertisers in the Journal Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.



XENOPUS LAEVIS TOAD

Pregnancy Diagnosis

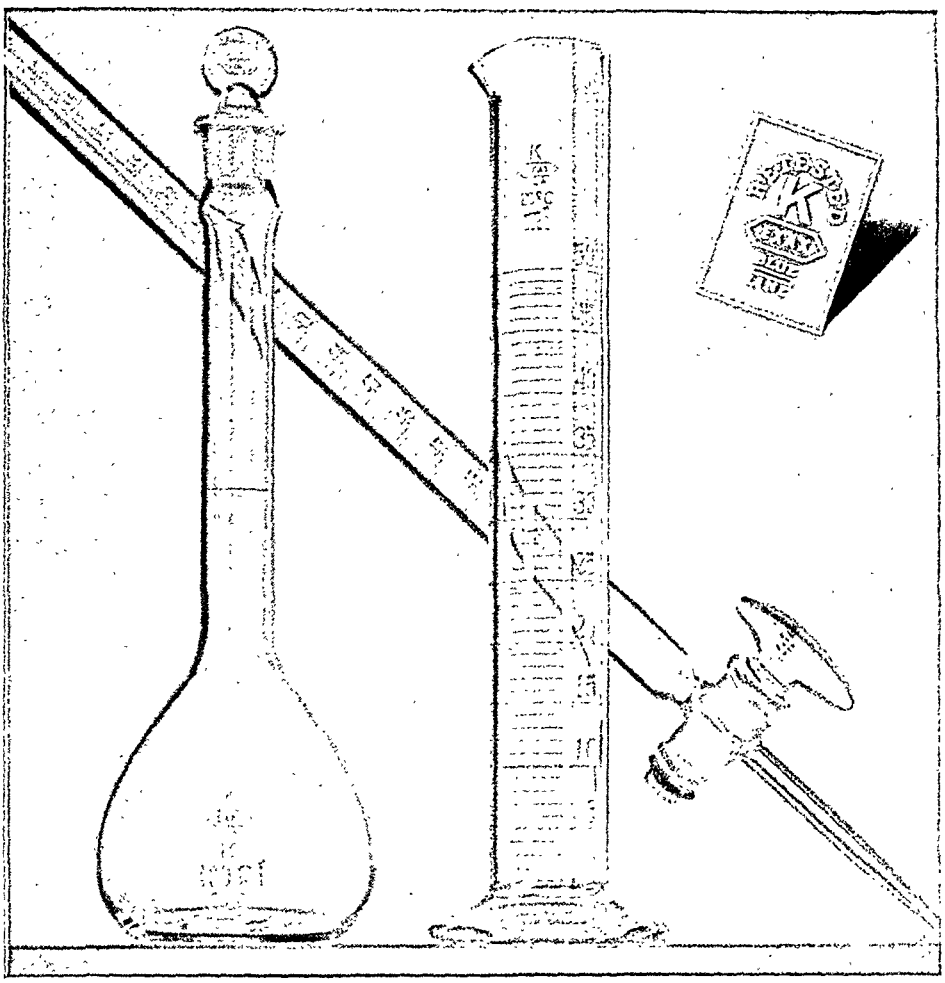
Animal Costs Greatly Reduced

We assure you of a continuous supply. Accurate, speedy and inexpensive. Average animal cost per test, 20 cents to 35 cents. We carry a complete stock of Laboratory Tanks, Isolation Jars, Holders, and correct reagents and apparatus for running tests. Speedy delivery via AIR EXPRESS. Current brochure showing new low prices sent upon request.

Jay E. Cook, Importer

P. O. Box 302 Baltimore 3, Md., U.S.A.
Cable Address—XENOPUS-BALTIMORE

(In writing to advertisers, please mention the Journal—it helps.)



Kimble EXAX RETESTED Flask No. 28015; Cylinder No. 20030; Burette No. 17056.

There's no substitute for EXAX RETESTED quality

EXAX RETESTED is America's No. 1 choice for accurate laboratory determinations simply because it is made and intended for that purpose. EXAX won't bounce, but it is thoroughly annealed for toughness . . . will stand everyday handling and usage.

The great value of EXAX RETESTED lies in certain exclusive advantages. It is a special formula of glass, precision-made by specialty craftsmen.

The fine deep graduations are of a type which readily accepts the permanent fused-in blue filler. *And* . . . all calibrations are marked and checked and *retested* with standards of highest precision.

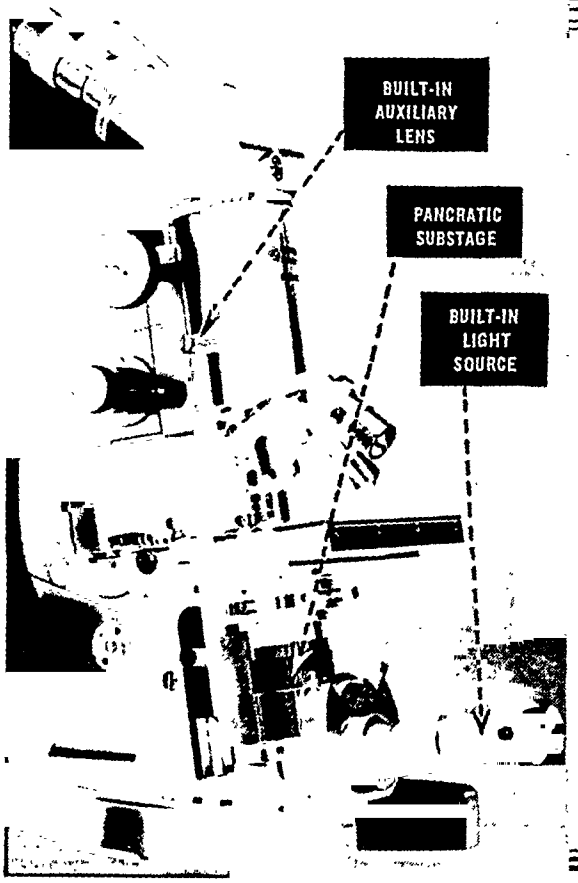
So remember, if accuracy and fitness for the job are important, there is no substitute for EXAX RETESTED glassware.

Look for the Kimble "K", the visible guarantee of invisible quality.

KIMBLE GLASS TOLEDO 1, OHIO

Division of Owens-Illinois Glass Company

(In writing to advertisers, please mention the Journal—it helps.)



GALILEO CORP. OF AMERICA, Inc.

Announces

The VNP Phase-Contrast Microscope with features which open completely new possibilities in phase-contrast technique.

•

For descriptive brochure write

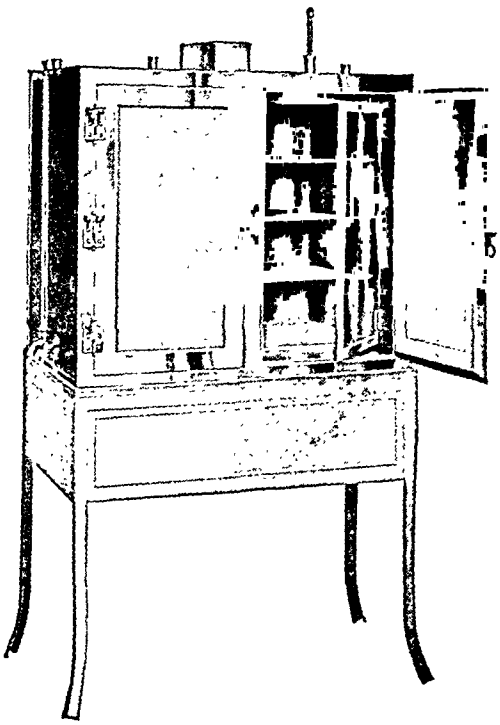
PFALTZ & BAUER, Inc.
Empire State Building, New York 1, N. Y.
Sole agents for
GALILEO CORPORATION OF AMERICA, Inc.
745 Fifth Avenue, New York 22, N. Y.
Distributor for Officine Galileo, Italy
Florence • Milan • Venice

Since 1881 manufacturers of research microscopes and microscopical equipment of all kinds, refractometers, polarimeters, and analytical balances.

NOW! MONEL METAL

adds Years to the Service of

***Castle* INCUBATORS**



Castle's proved performance now has a new advantage in construction—Monel throughout.

This lifetime metal eliminates pitting and porosity that often appear in copper (formerly the standard metal in incubators) from the action of chemicals now found in water systems.

Precise performance is essential for bacteriological work . . . and precision is the outstanding feature of Castle Incubators:

CONSTANT TEMPERATURE, with ¼° to ½°C. ± variation during the incubating period, despite changes in room temperature;

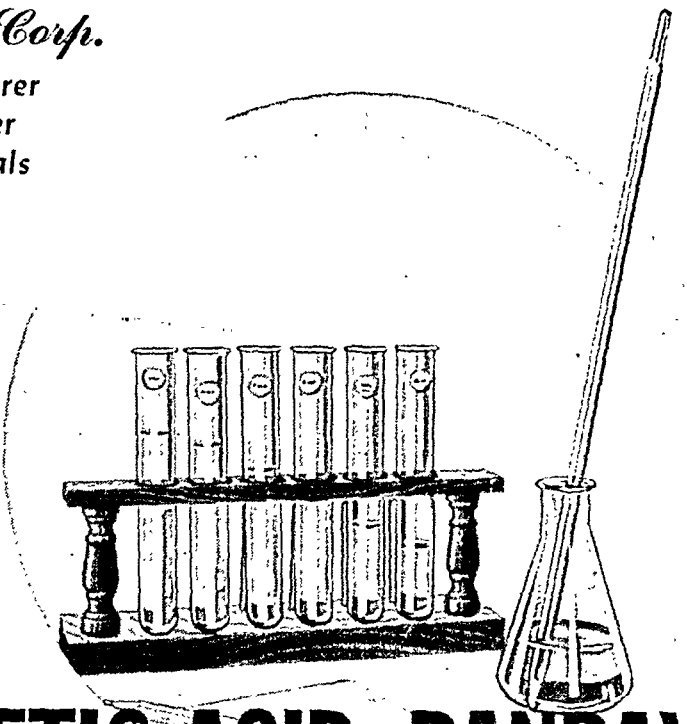
UNIFORM TEMPERATURE, with no more than ⅞°C. ± variation between top and bottom of the chamber *when loaded.*

For full details, see your Castle dealer, or write: Wilmot Castle Co., 1216 University Ave., Rochester 7, New York.

***Castle* BACTERIOLOGICAL APPARATUS**

The Panray Corp.

pioneer manufacturer of DOPA and other specialized chemicals for investigation and research...



announces the availability of...

IODOACETIC ACID, PANRAY

TWICE RECRYSTALLIZED, SPECIAL REAGENT GRADE

for the Iodoacetate Index Determination of Serum Proteins

DOPA "PANRAY"

(3,4-dihydroxyphenylalanine, C. P.)

available in l and dl forms

Synthetic chemical reagents ... capable of revealing the presence of an oxidase in cells elaborating melanin. Valuable indicators of active melanogenesis.

Dr. Huggins and his associates have devised a new, modified technique for the determination of *deficient thermal coagulation of serum proteins.*

Write for *your* copy of this important addition to cancer research literature.

Write for prices and other pertinent data

The Panray Corp.

340 CANAL ST.
NEW YORK 13, N. Y.

Custom Manufacturers of Fine Organic Chemicals

DIAGNOSIS OF VIRAL AND RICKETTSIAL INFECTIONS

Frank L. Horsfall, Jr., M.D., *Editor*

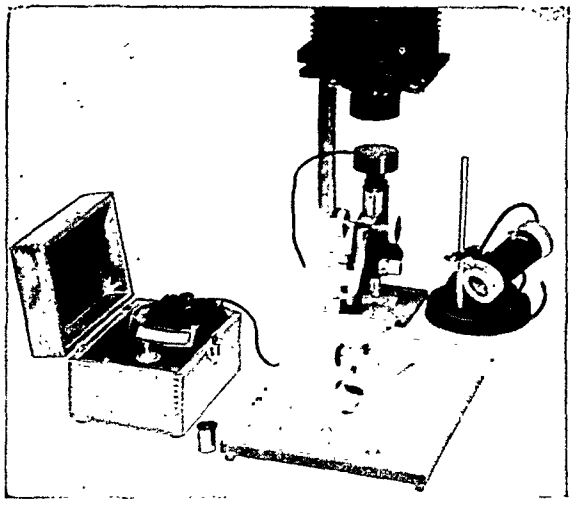
The latest information on laboratory diagnosis of each of a total of 32 specific infectious diseases caused either by viruses or by rickettsiae. *Symposia of the Section on Microbiology, New York Academy of Medicine, No. 1.* $3.75

EVALUATION OF CHEMOTHERAPEUTIC AGENTS

Colin M. MacLeod, M.D., *Editor*

A general evaluation of certain of the many factors involved in the success or failure of chemotherapy. ". . . an excellent review of the most recent and authoritative opinions about the various chemotherapeutic and antibiotic agents." —ARCHIVES OF PHYSICAL MEDICINE. *Symposia of the Section on Microbiology, New York Academy of Medicine, No. 2.* $4.00

COLUMBIA UNIVERSITY PRESS
2960 Broadway, New York 27



PHOTOVOLT

Exposure Photometer Mod. 200-M

for

PHOTOMICROGRAPHY

A photoelectric instrument of high accuracy and sensitivity, dependable and simple in operation, designed to measure light at microscope exit for determination of exact exposure time in black-and-white as well as color photomicrography **$65.—**

Also: **Electronic Photometers Mod. 500-M and 512-M**

for
- light and color readings on small areas in focal plane
- quantitative histochemical and cytochemical analysis
- measurement of lowest light values with photo-multiplier

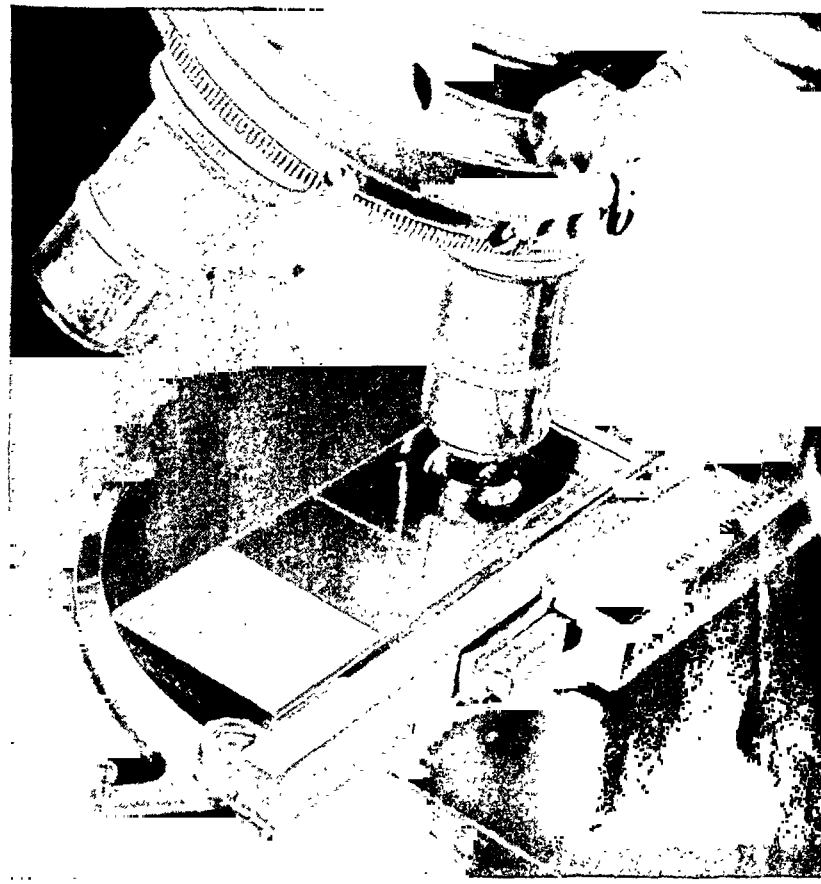
Write for Circular #810 and for 22-page Technical Information Bulletin #812

PHOTOVOLT CORP.

95 Madison Ave. — Clinical Colorimeters, Fluorescence Meters, Hemoglobinometers, pH Meters, Densitometers — New York 16, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)

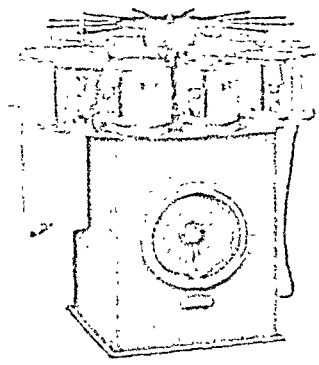
as dependable as the morning mail . . .



There they are, waiting for you each morning . . . all yesterday's tissues *automatically processed overnight by your own selected technique.* Dependable, day-in, day-out schedule . . . and normal routine in **Autotechnicon**-equipped laboratories the world over. **Autotechnicon** is versatile . . . it fixes, dehydrates, washes, infiltrates, stains . . . delivers beautifully finished tissues of superb diagnostic potential . . . with the *plus* factors of economies in *time, labor, materials.* A brochure describing **Autotechnicon** is available. May we send it?

Autotechnicon

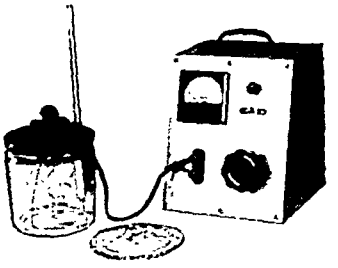
automatic tissue processing by any histologic technique



THE TECHNICON COMPANY

215 EAST 149th STREET • NEW YORK 51, N. Y.

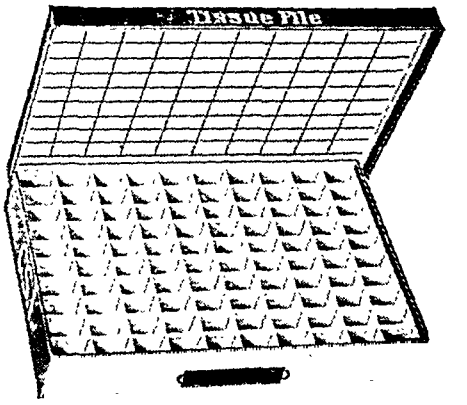
Eberbach
BONE DECALCIFIER



Decalcifies bone rapidly by electrolysis. This latest procedure preserves soft tissue patterns and staining properties better than slow acid immersion techniques which require days. Decalcifier, without electrodes, sells for only $75.00. Request Bulletin 180.

Eberbach & Son Company
Laboratory Apparatus & Supplies
Ann Arbor, Mich.

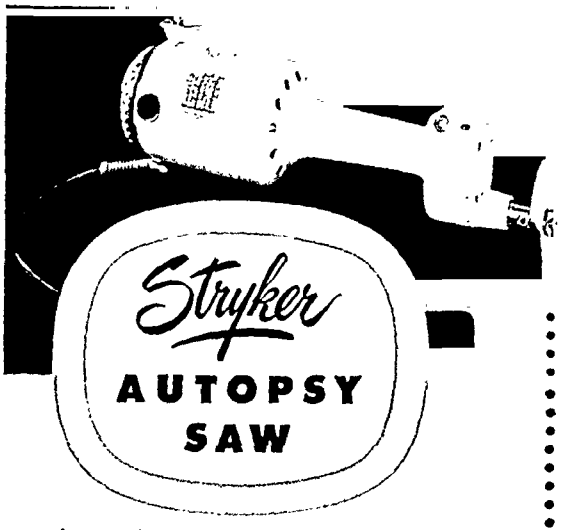
The Cargille
TISSUE FILE



Very convenient for filing and storing Histology Tissues Embedded in Paraffin or Celloidin Blocks.

Write for leaflet TF

CARGILLE SCIENTIFIC INC.
118 Liberty Street New York 6, N.Y.

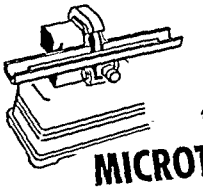


Stryker
AUTOPSY SAW

A new instrument which simplifies bone cutting

Electrically driven, oscillates at high speed to cut bone efficiently with complete safety. Cutting blades do not hurl material. Two-sided blade can be adjusted to three positions. Blade, arbor and shaft are stainless steel. Write Department C for complete information.

ORTHOPEDIC FRAME COMPANY Kalamazoo Michigan



the Berkeley
MICROTOME KNIFE SHARPENER
assures an
INCOMPARABLE EDGE
3 times faster...



COMPARE THE EDGES. Photomicrograph *(a)* shows a microtome knife edge sharpened by the Berkeley sharpener. Note its even smoothness, assurance of better sections. Photomicrograph *(b)* shows a blade laboriously hand sharpened, honed and stropped, but note the nicks and serrations still remaining.

COMPARE THE TIME. In tests, wherever made, and in practice in hundreds of laboratories, the Berkeley Microtome Knife Sharpener gives perfect edges in one-third the time required for hand honing and stropping.

Let us send you Bulletin MKS-14 giving full information about this indispensable laboratory aid.

Berkeley Scientific Company
SIXTH AND NEVIN AVE • RICHMOND, CALIFORNIA

for

BLOOD TYPING
RH TESTING

you need serum of the highest titer available.

ANTI-HUMAN SERUM (rabbit)
for the Anti-Globulin Test (Coombs')

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human Red Cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic Serum
for control tests

P.S.A.
(Paraffin Section Adhesive)

Write for complete information.

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York

1/2 Price Sale

In order to reduce our inventories, the following titles are offered at one-half the original price:

Malaria: Its Diagnosis, Treatment and Prophylaxis

By William Newbold Bispham, M.D.

Presents in eminently simple and practical form all the necessary information concerning malaria.
205 pp., 4 color plates. Was $3.50
Now $1.75

Etiology, Diagnosis and Treatment of Amebiasis

By Charles Franklin Craig, M.D.

A masterly treatise presenting a vast amount of essential data on amebiasis.
340 pp., 45 figs. Was $4.50 Now $2.25

Essentials of Clinical Allergy

By Samuel J. Taub, M.D.

A clear, clinical approach to the various allergic diseases as seen in practice.
208 pp., 2 figs., 16 plates. Was $3.00
Now $1.50

Nasal Medication

By Noah D. Fabricant, M.D.

A practical guide to the facts of modern nasal therapy, ready for application.
143 pp., 20 figs. Was $2.50 Now $1.25

Lead Poisoning

By Abraham Cantarow, M.D., and Max Trumper, Ph.D.

Presents in detail the problems common to all lead industries, and analyzes every phase of lead poisoning.
277 pp., 4 figs. Was $3.00 Now $1.50

Pathology of the Oral Cavity

By Lester Richard Cahn, D.D.S.

Covers the conditions that are likely to be encountered in everyday practice. Emphasizes clinical applications.
249 pp., 165 figs. Was $5.50 Now $2.75

Experimental Catatonia

By Herman H. deJong, M.D.

Represents stimulating research by an internationally known scientist. Skillfully written and authoritative.
239 pp., 38 figs. Was $4.00 Now $2.00

Sir W. Arbuthnot Lane, His Life and Work

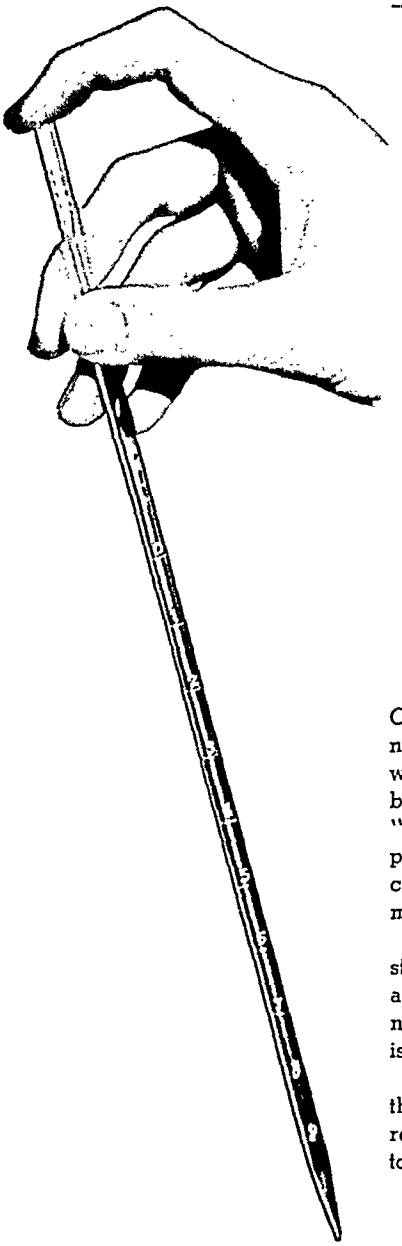
By W. E. Tanner, F.R.C.S.

Interesting and readable biography, with side lights on the famous Guy's Hospital, London.
200 pp. Was $4.50 Now $2.25

Orders should be sent direct to the publisher

THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves. Baltimore 2, Maryland

(In writing to advertisers, please mention the Journal—it helps.)



T.M. REG. PYREX U.S. PAT. OFF. BRAND

PIPETTES...

Quickly readable, unaffected by repeated sterilization

Clinical research makes many demands on the technicians in charge. Speed is one of them. But speed without accuracy avails nothing. That's why PYREX brand Pipettes are designed for easy readability. The "Lifetime Red" graduations are permanent, part of the pipette body itself. "Lifetime Red" will not wear or chip off in long service. Tips are fabricated to give maximum protection from chipping.

Made from PYREX brand glass No. 7740, they withstand repeated sterilization (wet or dry). They are unaffected thermally or chemically by any of the methods normally employed in laboratories where sterilization is a major factor in routine or in specialized work.

The measuring and serological types more than meet the tolerances of Federal Specification D.D. V-581 in regard to accuracy limits. When you call your laboratory supply dealer, ask for PYREX brand Pipettes!

Stocked by Leading Laboratory Supply Houses

CORNING means research in GLASS

PYREX

CORNING GLASS WORKS • CORNING, N. Y.

LABORATORY GLASSWARE

TECHNICAL PRODUCTS DIVISION: LABORATORY GLASSWARE • SIGNALWARE • GLASS PIPE • GAUGE GLASSES • LIGHTINGWARE • OPTICAL GLASS • GLASS COMPONENTS

(In writing to advertisers, please mention the Journal—it helps.)

THE BEST ALL-PURPOSE MEDIUM

DEXTROSE PHOSPHATE BROTH

AT THE LOWEST PRICE
$5.00 LB.

SAMPLE AND LITERATURE ON REQUEST

ALBIMI LABORATORIES, Inc.
16 CLINTON STREET
BROOKLYN 2, N. Y.

NEW THIRD EDITION

A Textbook of

CLINICAL PATHOLOGY

Edited by the late
Francis P. Parker, M.D.

Formerly *Kracke and Parker*, the present edition has been almost entirely rewritten. New procedures developed in the last six years have been added, and rearrangement of sequence in some sections has improved the continuity.

Emphasis on interpretation of laboratory findings
Contributors all experienced workers
Wide range of topics in relatively small compass

Comprehensive Accurate Lucid

1043 pp., 229 figs., 46 plates, $9.00

The Williams & Wilkins Co.
Mt. Royal & Guilford Aves., Baltimore 2, Md.

BBL

TRYPTICASE SOY BROTH

An all-purpose fluid medium, suitable for blood cultures and for the testing of antibacterial action.

Prices and literature sent on request

Dept. 501

BALTIMORE BIOLOGICAL LABORATORY
INCORPORATED

1640 Gorsuch Avenue Baltimore-18, Md.

Reagents for the Davidsohn Differential Test for Infectious Mononucleosis

BOILED GUINEA PIG KIDNEY ANTIGEN
(Desiccated)

BOILED BEEF CELL ANTIGEN
(Desiccated)

6 Individual Test Ampuls
(0.5 cc each).....$4.00
1 cc..... 1.00
2.5 cc..... 2.00
5 cc..... 3.00

SHEEP CELLS
Can be prepared from the following:

WASHED POOLED SHEEP CELLS
Stabilized 10% Suspension
A simple dilution prepares the 2% suspension
30 cc.....$1.50

STERILE SHEEP BLOOD (Alsever's)
(Equal parts of blood and modified Alsever's solution)
15 cc.....$1.50

STERILE SHEEP BLOOD (Citrated)
15 cc.....$1.50

CAPPEL LABORATORIES
Diagnostic Reagents
Box 352 Wayne, Pa.



Books make excellent gifts—and nothing is more appropriate for a friend or associate in medicine than one to help him in his work.

Choose your Christmas book-gifts from the outstanding Mosby titles listed below. We will enclose a Christmas Greeting card with your name and ship direct from here. Just tell us "who" and "where" in time to get it off before the Christmas rush

—— *1949 Releases* ——

★ ATLAS OF AMPUTATIONS
By DONALD B. SLOCUM, M.D., M.S., 584 pages, 564 illustrations, Size: 8½ x 11-in. $20.00

★ ATLAS OF OBSTETRIC TECHNIC
By PAUL TITUS, M.D., SECOND EDITION. 197 pages, illustrated. $7.50

★ DOCTOR AND PATIENT AND THE LAW
By LOUIS J. REGAN, M.D., LL.B., SECOND EDITION. 590 pages, $10.00

★ Essentials of OBSTETRICAL AND GYNECOLOGICAL PATHOLOGY
By ROBERT L. FAULKNER, M.D., F.A.C.S., and MARION DOUGLASS, M.D. SECOND EDITION. 400 pages, 300 illustrations, 3 in color. $8.75

★ *Handbook of* DIGESTIVE DISEASES
By the late JOHN L. KANTOR, M.D., F.A.C.P. and ANTHONY M. KASICH, M.D., F.A.C.P. SECOND EDITION. 658 pages, 149 illustrations, 2 in color. $11.00

★ Handbook of DISEASES OF THE SKIN
By RICHARD L. SUTTON, M.D., and RICHARD L. SUTTON, JR., M.D., 749 pages, 1057 illustrations. $12.50

★ ATLAS OF ROENTGENOGRAPHIC POSITIONS
By VINITA MERRILL. IN TWO VOLUMES. 708 pages, over 1500 illustrations. Size: 9 x 12-in. $30.00

★ OCULAR SIGNS IN SLIT-LAMP MICROSCOPY
By JAMES HAMILTON DOGGART, M.A., M.D., F.R.C.S. (Eng.) 112 pages, 93 illustrations, 85 in color. $6.75

★ OPERATIVE ORTHOPEDICS (*Campbell's*)
Edited by J. S. SPEED, M.D., and HUGH SMITH, M.D., et al. SECOND EDITION. IN TWO VOLUMES. 1600 pages, 1141 illustrations, 2 in color. $30.00

★ THE PRACTICE OF REFRACTION
By SIR W. STEWART DUKE-ELDER, K.C.V.O., D.Sc., Ph.D., M.D., (Eng.) 316 pages, 216 illustrations. $6.25.

★ THE DIAGNOSIS AND TREATMENT OF BRAIN TUMORS AND THE CARE OF THE NEUROSURGICAL PATIENT
By ERNEST SACHS, A.B., M.D., SECOND EDITION. 626 pages, 345 illustrations, 10 in color. $15.00

★ *Handbook of* MATERIA MEDICA, TOXICOLOGY AND PHARMACOLOGY
By FORREST RAMON DAVISON, B.A., M.Sc., Ph.D., M.B., FOURTH EDITION. 730 pages, 35 illustrations, 4 in color. $8.50

To be ready for Christmas! *FROM THE HILLS* An Autobiography of a Pediatrician By John Zahorsky, M.D.

This is the story of Dr. Zahorsky's life memoirs of a happy doctor told in the same simple style which has characterized his medical writings. 350 pages. Price $4.00.

THE C. V. MOSBY COMPANY

Scientific Publications

3207 Washington Blvd.
St. Louis 3, Missouri

722 Post Street
San Francisco 9, California

(In writing to advertisers, please mention the Journal—it helps.)

Since 1928

GUINEA PIGS

RABBITS

(Friedman Does)

WHITE MICE

Satisfactorily shipped 1000 miles and more

By

HILLTOP CAVIARY

Box 195 **Scottdale, Pa.**

Phone 8113R12

★

When writing for quotations or other information, please indicate weight, sex and NUMBER of animals you require.

POLYPHIL
Sheep Red Cells

Uniformity and dependability in the complement-fixation field have long been retarded through the use of variable reagents. Inconsistency in cell resistance has been the cause of many unsatisfactory complement-fixation tests. To help eliminate this condition, Carworth Farms is pleased to announce the production of their new and improved POLYPHIL Sheep Red Cells.
Other serological and bacteriological products available are . . .

VACSEAL COMPLEMENT
STERILE SHEEP BLOOD • RABBIT BLOOD
LYOPHILIZED RABBIT PLASMA

A trial package of any of the above products will be sent to laboratories on request.

CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK

World-Wide Abstract Service

EXCERPTA MEDICA

An International Medical Abstract Service published in English and covering the whole field of Clinical and Experimental Medicine

I. Anatomy, Anthropology, Embryology and Histology	$22.50
II. Physiology, Biochemistry and Pharmacology	45.00
III. Endocrinology	15.00
IV. Medical Microbiology and Hygiene	22.50
V. General Pathology and Pathological Anatomy	37.50
VI. Internal Medicine	37.50
VII. Pediatrics	15.00
VIII. Neurology and Psychiatry	22.50
IX. Surgery	25.00
X. Obstetrics and Gynecology	15.00
XI. Oto-rhino-laryngology	15.00
XII. Ophthalmology	15.00
XIII. Dermatology and Venereology	25.00
XIV. Radiology	15.00
XV. Tuberculosis	15.00

Special rate to libraries ordering all 15 sections $250.00

Published monthly, one volume a year. Subscription by volume only, payable in advance. Send for a sample copy of the section which covers your specialty

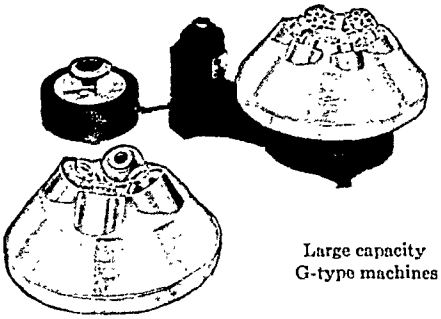
THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves.
Baltimore 2, Md.

Sole distributor for the United States, Canada and Central America

SERVALL

ANGLE CENTRIFUGES

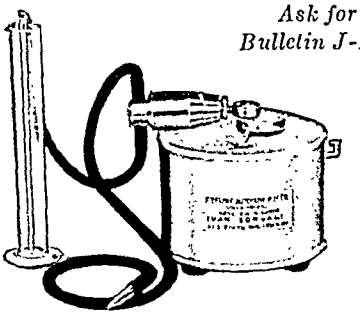
Ask for Bulletin J-121
with complete data on all models



Large capacity
G-type machines

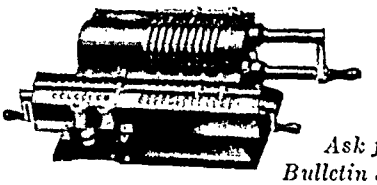
Type G/1, for 6 bottles of 250 cc each
18 tubes of 60 cc each
42 tubes of 15 cc each
78 tubes of 10 cc each
Type G/2, for 4 bottles of 500 cc each
Type G/3, for 10 tubes of 100 cc each

Ask for
Bulletin J-122



STERLING AUTOMATIC
AND
HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.
Easy to operate—Time-Saving



Ask for
Bulletin J-123

ORIGINAL—ODHNER

Portable Calculator for the Scientist
Many different models
Efficient - Sturdy - Low Priced

Manufacturers and Distributors:

IVAN SORVALL, Inc.
210 FIFTH AVE.-NEW YORK 10, N. Y.

HELLIGE
SOLUTIONS
AND
REAGENTS



A complete line of solutions and reagents is available at reasonable prices for bio-chemical tests with

HELLIGE-DILLER PHOTOELECTRIC COLORIMETERS

and

HELLIGE-DUBOSCQ COLORIMETERS

Hellige solutions and reagents are scientifically prepared from chemicals of the highest quality. Through our special service department, orders are shipped the day they are received.

FOR GUARANTEED RESULTS
ALWAYS SPECIFY THE HELLIGE PRODUCT

Catalogs on Request

HELLIGE
INCORPORATED
3718 NORTHERN BLVD., LONG ISLAND CITY 1, N.Y.

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia...O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 1216 Pennsylvania Ave., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

American Society of Clinical Pathologists

OFFICERS

President: Dr. O. A. Brines, Wayne University College of Medicine, Detroit 26, Michigan
Vice-President: Dr. C. H. Manlove, 5725 S.E. Stark Street, Portland 15, Oregon
President-Elect: Dr. J. B. McNaught, University of Colorado School of Medicine, Denver, Colorado
Secretary-Treasurer: Dr. C. G. Culbertson, 6060 Park Avenue, Indianapolis 5, Indiana

STANDING COMMITTEES

EXECUTIVE COMMITTEE

W. S. Thomas, Chairman, Rochester, N. Y.
T. J. Curphey, Long Island, N. Y.
F. W. Konzelmann, Atlantic City, N. J.
L. W. Larson, Bismarck, N. D.
S. P. Reimann, Philadelphia, Pa.
J. R. Schenken, Omaha, Neb.

BOARD OF CENSORS

L. W. Diggs, Chairman, Memphis, Tenn.
J. J. Andujar, Fort Worth, Tex.
F. C. Coleman, Des Moines, Iowa
J. W. Howard, Wilmington, Del.
V. M. Johnson, West Palm Beach, Fla.
C. P. Larson, Tacoma, Wash.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. Montgomery, Chairman, Muncie, Ind.
A. G. Foord, Pasadena, Cal.
F. B. Lynch, Jr., Philadelphia, Pa.
F. B. Queen, Portland, Ore.
R. G. Stillman, New York, N. Y.
A. H. Wells, Duluth, Minn.

APPOINTED COMMITTEES 1948-1949

BOARD OF CONSULTANTS FOR ENLARGING TUMOR REGISTRY

P. Klemperer
S. Otani*
S. P. Reimann
A. R. Rich*
F. W. Stewart*
A. P. Stout*
S. Warren
* Non-members

COMMITTEE ON EDUCATION AND PUBLICITY

W. O. Russell, Chairman
J. H. Black
M. Fein
W. M. Germain
D. A. Nickerson
S. P. Reimann

COMMITTEE ON NECROLOGY

H. R. Brown

COMMITTEE ON REGISTRY AND LOAN COLLECTIONS

F. J. Heck—Hematologic Division
J. W. Kernohan—Pathologic Division
E. S. Moss—Parasitologic Division

COMMITTEE TO STANDARDIZE EDUCATION AND TRAINING OF MEDICAL TECHNOLOGISTS

J. J. Andujar, Chairman
F. C. Coleman
O. O. Christianson
R. M[illegible]er
E. Moss
M. Oosting
P. Rosenblatt
J. P. Tollman

COMMITTEE ON SEMINAR

I. Davidsohn, Chairman
P. R. Cannon
Gen. R. O. Dart
R. A. Moore

REPRESENTATIVES ON NATIONAL RESEARCH COUNCIL SUBCOMMITTEE ON ONCOLOGY

I. H. Perry
S. Warren

GENERAL RESEARCH COMMITTEE

M. Strumia, Chairman
A. H. Sanford
W. M. Simpson

PROGRAM COMMITTEE

C. G. Culbertson, Chairman
R. A. Moore, Co-Chairman
C. J. Bucher
F. B. Queen
F. W. Sunderman

COMMITTEE ON HEMATOLOGIC TERMINOLOGY

E. E. Osgood, Chairman
L. Berman
F. J. Heck
R. R. Kracke
L. R. Limarzi

REPRESENTATIVE ON BOARD OF GOVERNORS OF COLLEGE OF AMERICAN PATHOLOGISTS

T. J. Curphey

DIFCO

BACTO-THROMBOPLASTIN

for Prothrombin Time Determinations of Blood

A stabilized desiccated rabbit brain substance of uniformly high thromboplastic potency applicable to all prothrombin time procedures using blood or whole or diluted plasmas. It is especially recommended for use in following prothrombin activity in patients on dicumarol therapy.

Bacto-Thromboplastin is distributed in ampuls containing sufficient material for the preparation of approximately 3 ml. of thromboplastin extract. It is available in packages of six ampuls.

BACTO-CEPHALIN CHOLESTEROL ANTIGEN

for the Hanger Flocculation Test

An approved stable reagent for use in the Hanger Flocculation Test for determining the index of disturbance of the liver parenchyma.

Bacto-Cephalin Cholesterol Antigen is distributed in packages of six units, each unit containing the required amount of reagent to prepare 5 ml. of stock ether antigen solution and 150 ml. of final test antigen.

BACTO-THYMOL TURBIDITY REAGENT

for the Maclagan Thymol Turbidity Test

A standardized buffered thymol reagent recommended for use in the Maclagan Thymol Turbidity Test for indicating hepatic parenchymal impairment.

Bacto-Thymol Turbidity Reagent is distributed in packages of six bottles of 25 ml. each.

PHENOLSULFONPHTHALEIN AMPULS, DIFCO

for Renal Function Test

A sterile aqueous solution of the monosodium salt of Phenolsulfonphthalein standardized for parenteral use in renal function determinations. It is available in packages of 10 ampuls of 1 ml. each.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS

In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORARORIES

DETROIT 1, MICHIGAN

VOLUME 19 NOVEMBER 1949 NUMBER 11

AMERICAN JOURNAL OF CLINICAL PATHOLOGY

OFFICIAL PUBLICATION OF
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Agents: For the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Australia: Angus and Robertson, Limited, 89-95 Castlereagh Street, Sydney; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 NOVEMBER 1949 NUMBER 11

CONTENTS

Presidential Address: Not For Ourselves Alone. O. A. BRINES 999

The Incidence of Posttransfusion Serum Hepatitis. J. J. McGRAW, JR., M. M. STRUMIA AND E. BURNS 1004

The Coagulation Mechanism. With Specific Reference to the Interpretation of Prothrombin Time and a Consideration of the Prothrombin Consumption Time. A. J. QUICK 1016

Effect of Ultrafiltration and Carbon Dioxide on Antithrombin Activity of Fresh and Stored Human Plasma. M. Stefanini 1024

A Survey of the Accuracy of Rh Antibody Titrations in Several Hospital Laboratories. R. W. MARSTERS 1032

Interstitial Cell Tumor of the Testis. Report of Two Cases. C. R. REINERS, JR. AND R. C. HORN, JR. 1039

Book Reviews 1048

Selected Abstracts 1052

Technical Section

Automatic Tissue Dehydrating and Embedding Apparatus. J. TANNENBERG 1061

Paraffin Tissue-Block Trimmer. M. E. CONGER 1070

Critical Adsorption Levels and Electrophoretic Analyses of "Prothrombin-Free" Plasmas. S. GOLLUB, F. E. KAPLAN, D. R. MERANZE AND H. TUFT 1071

An Improved Apparatus for the Microdetermination of Cholesterol. E. M. ABRAHAMSON 1076

Penicillin-Resistant Staphylococci. W. G. RICE AND A. M. LONERGAN 1083

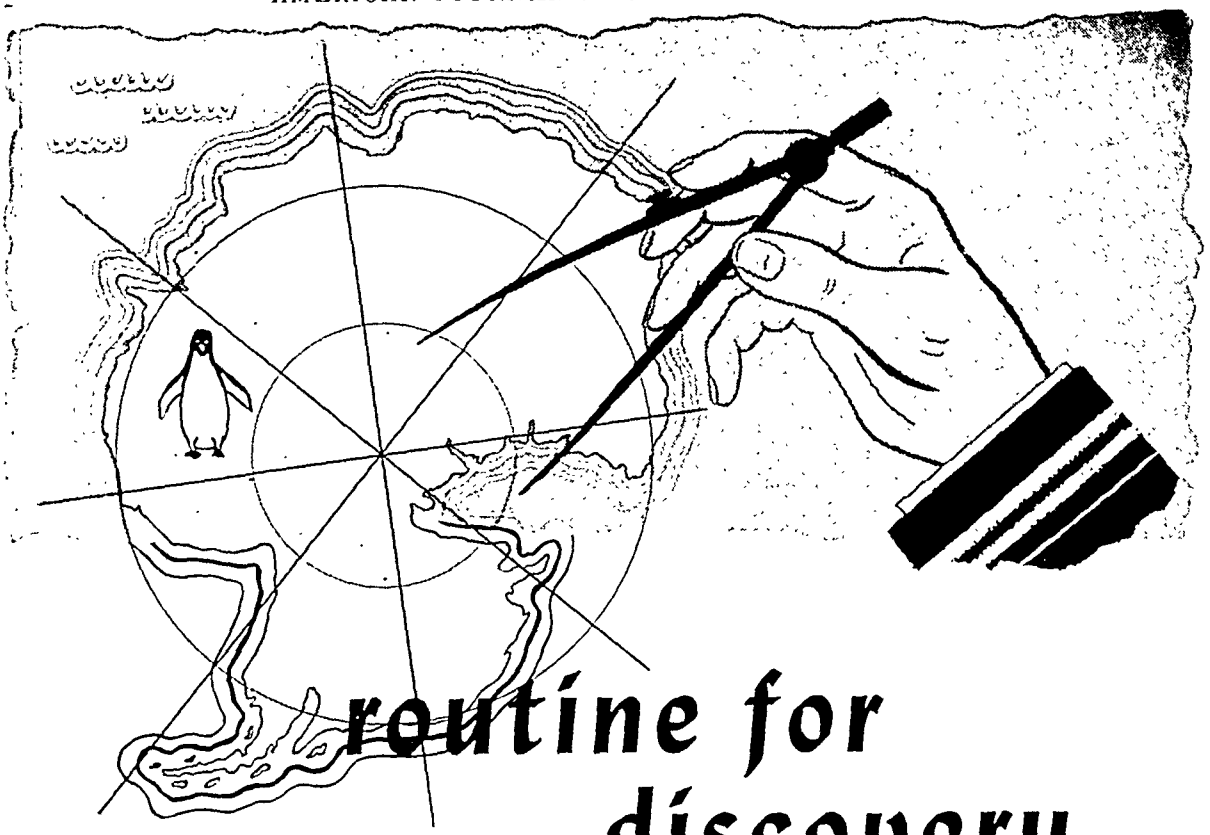
An Improvised Microtome Knife Sharpener. W. G. RICE 1089

Index of Subjects, Volume 19 1091

Index of Authors, Volume 19 1093

Table of Contents, Volume 19 iii

(In writing to advertisers, please mention the Journal—it helps.)



routine for discovery

The national total of undiagnosed or "unknown" diabetics may run from a million to two or even three.[1,2] Modern treatment, when promptly initiated, can do much to prevent metabolic decompensation and to minimize diabetic complications. Therefore, the clinical revealment of diabetes mellitus at an early stage is essential.

Thus, "all patients who present themselves to the physician for an examination should have a routine urine examination."[3] In this phase of practice, the advantages of *Clinitest*® tablets for urine-sugar analysis are considerable.

Clinitest is dependably accurate, yet it takes only a few seconds to perform. The test is simple—no external heat need be applied; interpretation is by direct color comparison. *Clinitest* is convenient both for the doctor's office routine and for the diabetic patient's prescribed sugar-level checkups.

(1) Joslin, E. P.: Postgraduate Med. 4:302 (Oct.) 1948. (2) Kemper, C. F.: Rocky Mountain M. J. 45:1092 (Dec.) 1948. (3) Pollack, H.: New York Med. 4:15 (Dec. 5) 1948.

Clinitest

for urine-sugar analysis

AMES

AMES COMPANY, INC. • ELKHART, INDIANA

(In writing to advertisers, please mention the Journal—it helps.)

First American Production of

Another New **National** *Stain*



THEDANE BLUE

SOLUTIONS T-3 AND T-5

for the Rapid and Precise Identification of Blood Parasites

Thedane Blues are very stable, tropic-proof solutions for demonstrating trypanosomes, malarial crescents, piroplasms, blood spirochetes, treponemes in syphilitic chancre exudates, leptospirae, leucocytes and reticulocytes. They assure rapid, reliable diagnosis by simultaneous hemolysis and monochromatic staining of the parasites. They have these advantages:

Save time and simplify procedure thru ultra-rapid staining of fresh blood preparations with undiluted stock solutions. 50 to 100 cu. m.m. (or more) of fresh blood can be prepared in less than 10 seconds.

Instantaneous hemolysis completely clears the image field. Only parasites, leucocytes and reticulocytes are stained.

Only medium or high dry systems are used. Large image fields assure rapid, reliable diagnoses even at lowest parasite densities.

In dark-field preparations, spirochetes and leptospirae show brilliantly red, indicating suspicious spots at medium power. High power verifies diagnosis.

Ideal staining for diagnosis of microfilariae in extremely thick wet blood drops at low power.

Indispensable in bird-malaria research because they make possible examination of thick drops in nucleated erythrocytes.

Very instructive for parasitological and hematological class room work.

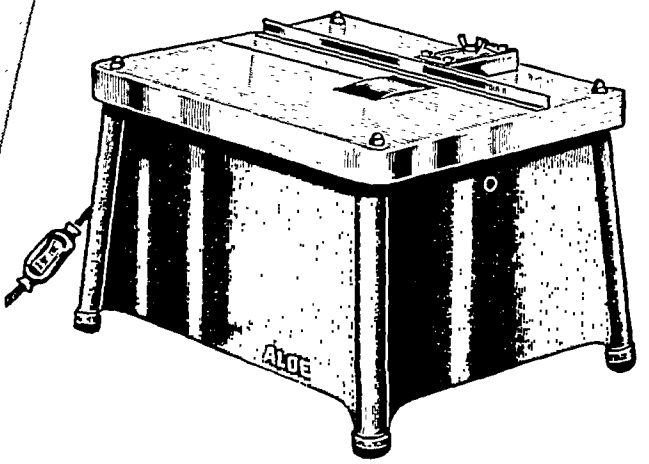
Leaflet giving detailed data on applicability and method of use sent on request.

Pharmaceutical Laboratories

NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A.

a better edge in less time!

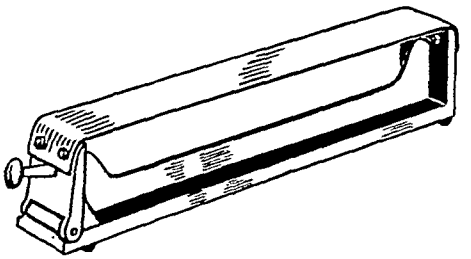


with the new **aloe**

electric microtome knife sharpener

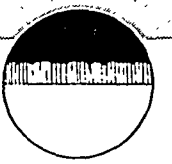
With the new, simplified Aloe Microtome Knife Sharpener the average technician is able to put a better, smoother, keener edge on microtome knives, scalpels and other instruments in far less time and with less effort. One reason for this quicker, more dependable service is the simplified knife holder that supports the knife over the full length up to 13½ inches. The holder is set to provide an optimum bevel for cutting cartilage, paraffin embedded tissue, bone, or soft metals such as lead.

The special alloy honing wheel is powered by a split phase, ¼ h.p. motor. Operation is unusually quiet and free from vibration. The entire mechanism is enclosed in a rubber-footed, crackle finish cast metal housing with an aluminum platform. Overall dimensions are 14¾" x 12" x 9½" high.

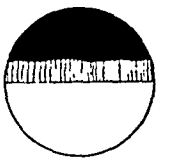


improved strop assembly

An improved strop assembly is now furnished as standard equipment with the Aloe Microtome Knife Sharpener. It removes the small wire or burr which accumulates on the knife during sharpening. The strop consists of a high quality horse-hide strop and a linen finishing strop mounted in a heavy metal frame, equipped with thumb screws for adjusting tension.



BEFORE SHARPENING *note ragged knife edge.*



FIRST HONING *removes nicks and rough spots after only a few strokes on honing wheel.*



COMPLETE HONING *gives fine edge but a fine wire or burr mars its keenness.*



AFTER STROPPING *cutting edge is smooth and even; uniformly sharp.*

60040—*Simplified Aloe Electric Microtome Knife Sharpener, as described, with adjustable strop assembly, one stick of abrasive paste, one jar of abrasive powder, one tube of special solvent, cord and plug. For operation on 115 volts, A.C.—each* **$250.**

a. s. aloe company *Serving Science Since 1860*

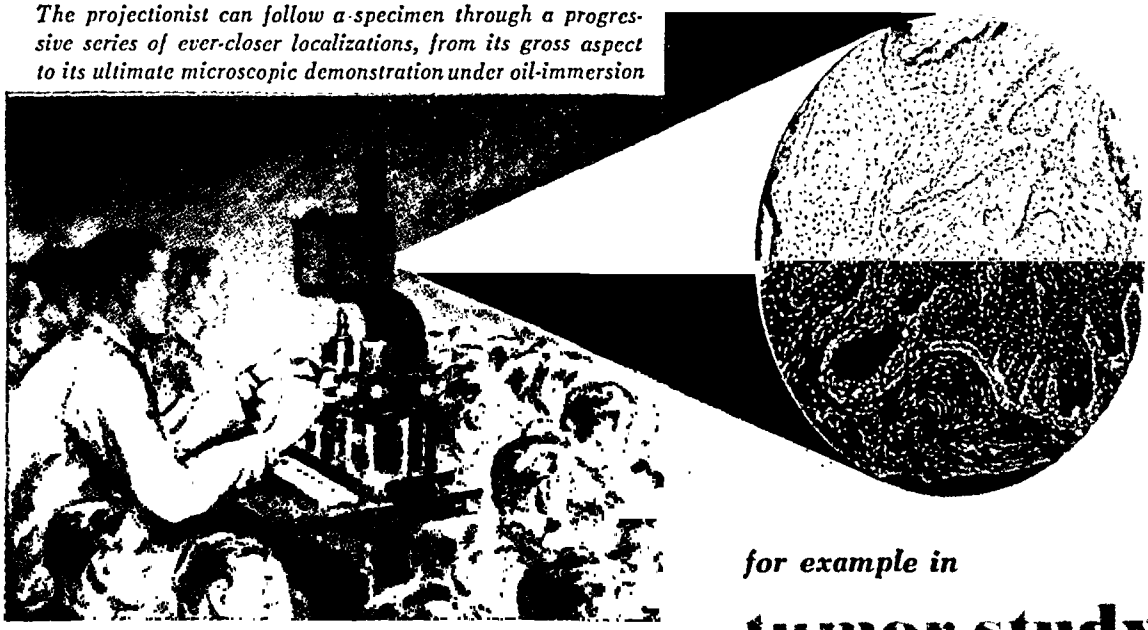
1831 Olive Street • St. Louis 3, Missouri

aloe

(In writing to advertisers, please mention the Journal—it helps.)

mass demonstration in the auditorium

The projectionist can follow a specimen through a progressive series of ever-closer localizations, from its gross aspect to its ultimate microscopic demonstration under oil-immersion

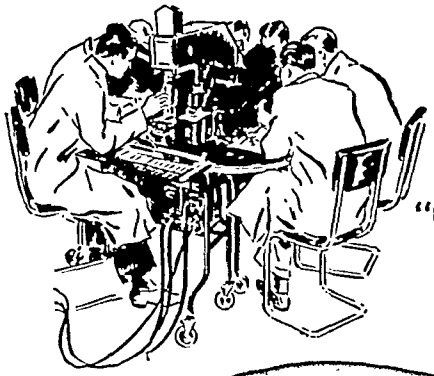


for example in

tumor study

The 64,000 lumen intensity of the Scopicon high-pressure mercury lamp permits auditorium demonstration of tissue sections even under oil-immersed microscope objectives. In projected images six feet or more across, every detail of the cellular structure stands out clearly, due to the pinpoint character of the 1 mm. square (approx.) focal spot employed. The Scopicon light is steady and flickerless . . . its brilliant white color exhibits biological stains to the greatest advantage. May we send you the brochure describing this versatile instrument?

The auxiliary Scopicon dark-chamber "round-table" affords superb facility for small-group study. Here the projected image is cast upon a platen within a light-tight chamber, with separate light-excluding viewing hoods for each observer. Any number up to ten may share in conference, which can be conducted in a normally lighted room. A pointer at each port permits any observer to indicate areas of interest to all the others for discussion.



. . . and for small-group "round-tables" in a normally lighted room

the Scopicon

microprojection photomicrography

SCOPICON, Inc.
215 East 149th St., New York 51, N. Y.

UNEQUALED

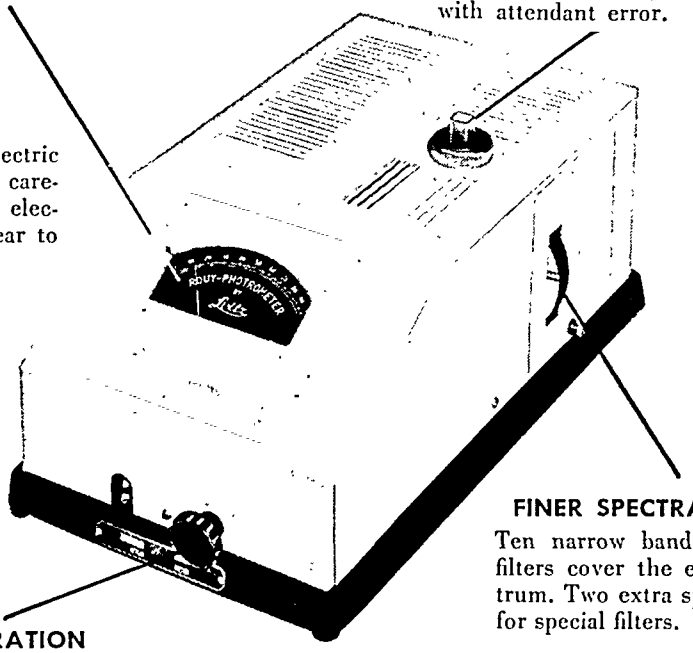
for accuracy • simplicity • dependability

the ROUY PHOTROMETER by Leitz

MORE ACCURATE READINGS
Only the Photrometer cuts functional error to within 0.1%.

GREATER OPTICAL ACCURACY
Precision square absorption cells with plano-parallel surfaces eliminate errors possible with ordinary curved cells. Optical system is designed to eliminate possibility of stray light with attendant error.

BETTER MATCHING OF ELEMENTS
Only Leitz matches photoelectric cell and microammeter so carefully that measurement of electrical energy is strictly linear to transmitted light energy.



FINER SPECTRAL DEFINITION
Ten narrow band (40 millimicron) filters cover the entire visible spectrum. Two extra spaces are provided for special filters.

EASIER OPERATION
All adjustments made with a single control. Any competent laboratory worker can use the Photrometer, for no special skills are required.

The Instrument for *ANY* Colorimetric Test—the Rouy Photrometer is pre-calibrated for the 38 most commonly required clinical tests and can be calibrated for *any test scientifically suited to colorimetric technique.*

No Standards . . . No Calculation—Pre-calibration and an Individual Handbook eliminate any need for calculation or preparation of standards. Accurate reagents are quickly available through the Leitz Solution Supply Service.

Performance Proved—Photrometers and Leitz Colorimeters are now serving more than 8,300 satisfied users. Each instrument is made to exacting standards of precision and sturdiness by Leitz, first large-scale producer of photoelectric colorimeters.

Pre-calibrated for 38 tests—$257.80
Pre-calibrated for 22 tests—$182.80

For information, write **E. LEITZ, Inc.**, 304 Hudson Street, New York 13, N. Y.

LEITZ SCIENTIFIC INSTRUMENTS • MICROSCOPES • BINOCULARS
LEICA CAMERAS AND ACCESSORIES

(In writing to advertisers, please mention the Journal—it helps.)

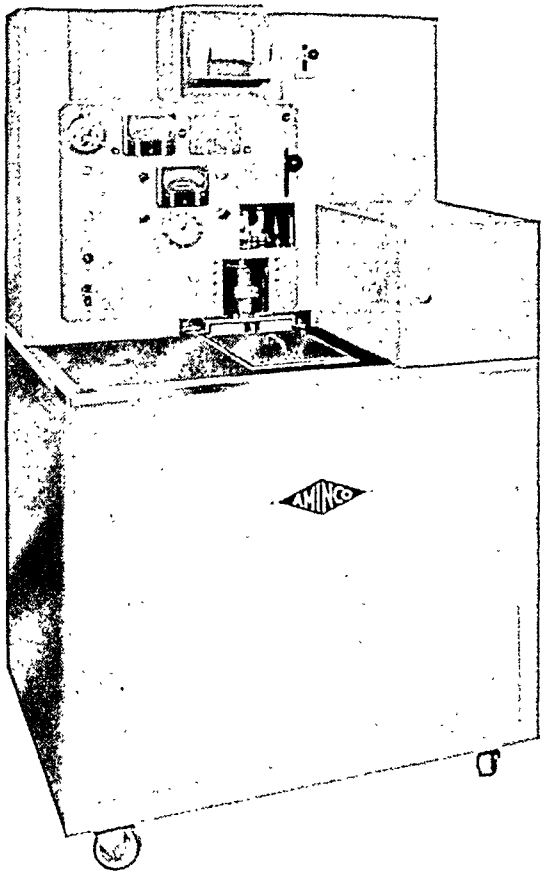
Far ahead of the field!

AMINCO STERN

ELECTROPHORESIS APPARATUS

it will pay you to investigate the MOST ADVANCED AND COMPLETE instrument for Electrophoretic analysis

Exclusive Features
NOT TO BE FOUND ELSEWHERE



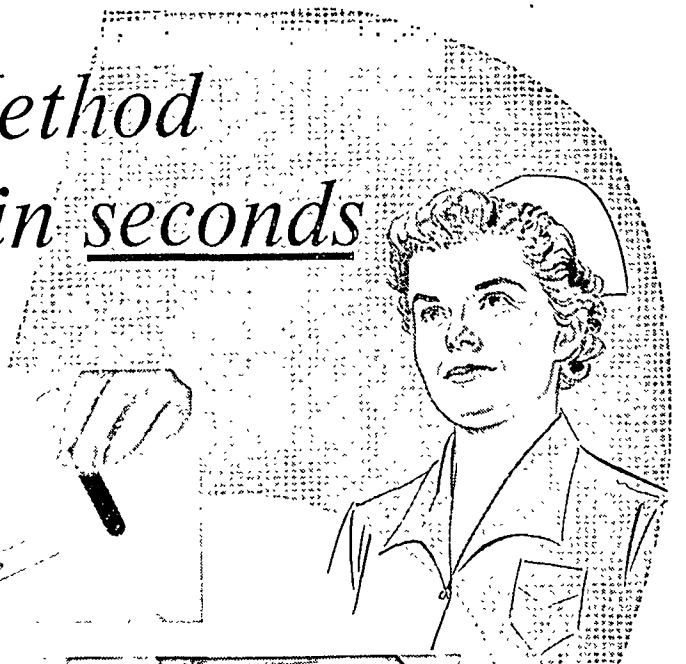
- Most economical because of greater analytical capacity.
- Most useful for research and routine work.
- High sensitivity . . . can detect protein concentrations as low as 0.002%.
- Operates continuously regardless of atmospheric conditions, except for servicing.
- Most complete . . . includes rapid dialysis facilities, chamber for freezing and storing specimens, sealed mechanical refrigeration system, precision Schlieren optics, complete photographic facilities, and a rotatable triple diaphragm for selecting slit, bar and straightedge patterns.
- Compact . . . measures only 30 x 37 x 67 inches high. Moved about with ease.
- More convenient . . . operator works in front of the instrument. All parts at convenient heights and locations.
- Shipped ready for operation.
- Accommodates semi-micro, macro, macropreparative, adsorption, and conductivity cells.
- Engineered for long life. Components rated far beyond their normal operating requirements.

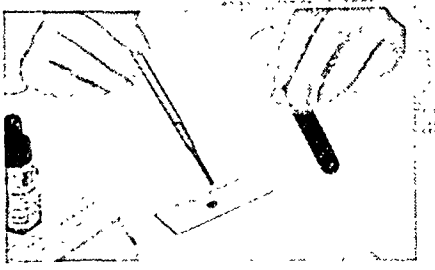
BULLETIN 2175-P
TELLS THE ENTIRE STORY
Write for your copy

Since 1919 AMINCO

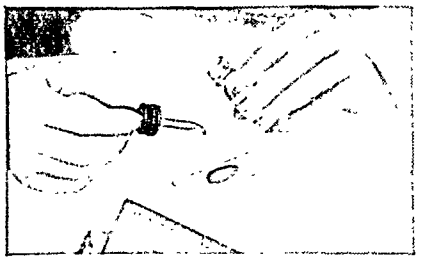
AMERICAN INSTRUMENT CO., Inc.
Scientific Instruments
SILVER SPRING, MARYLAND
IN METROPOLITAN WASHINGTON, D. C.

This Slide Method gives (Rh) test in seconds

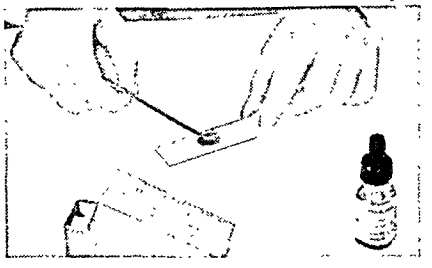




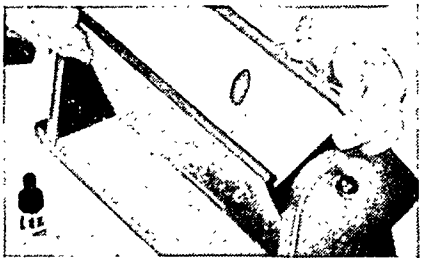
1 • *Place two drops of blood on slide. Fresh, clotted, oxalated, heparinized or citrated blood may be used.*



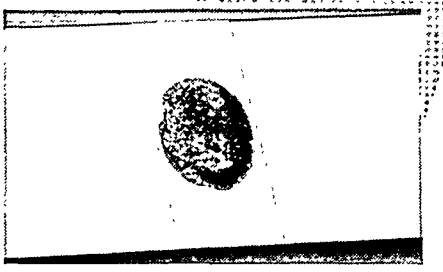
2 • *Add one drop of Anti-Rh serum.*



3 • *Mix Anti-Rh serum with blood and spread over slide.*



4 • *Place slide on view box. Tilt view box back and forth and watch for clumping. No microscope is needed.*



5 • *Clumping starts in 45 seconds, is complete in 1 to 2 minutes. Clumping indicates blood is Rh Positive; absence of clumping, Rh Negative.*

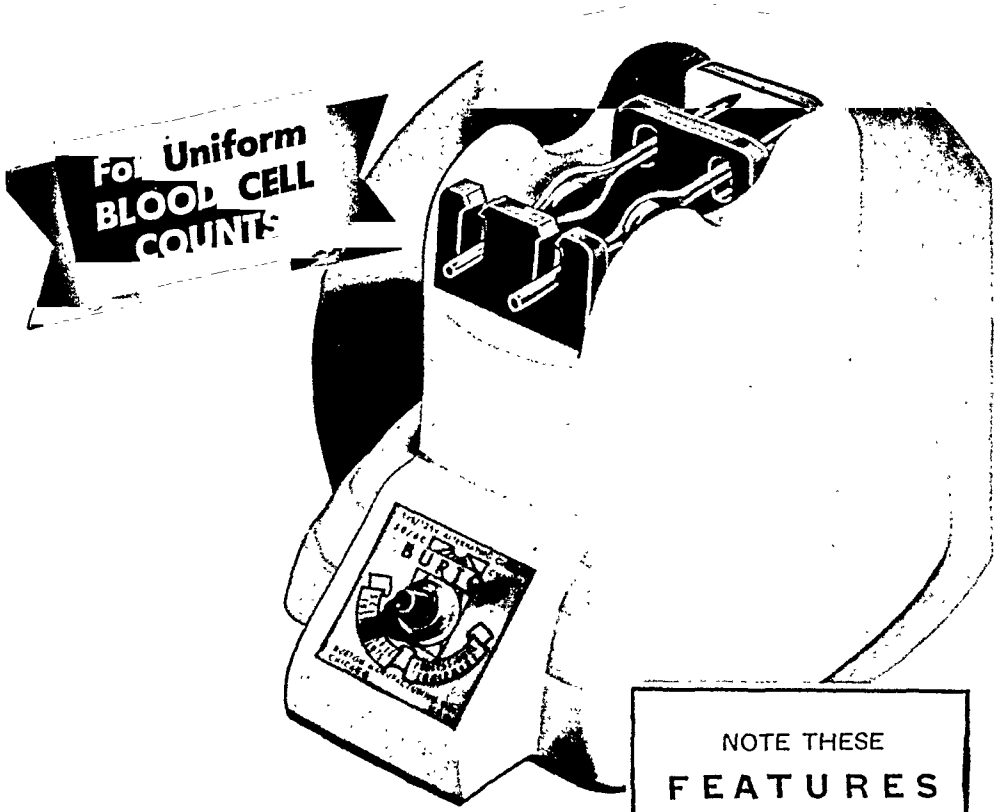
The Slide Method, using the view box, accounts in part for the rapidity and accuracy of Rh Tests. But even more important is the potency of the serums themselves.

Prepared under the strictest controls, and according to N. I. H. specifications, AMERICAN serums retain their original potency over a long period even under adverse conditions. Six strategically located distributing centers assure you of prompt service. Write today for complete descriptive literature.



PLAN WITH AMERICAN
... the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS
(In writing to advertisers, please mention the Journal—it helps.)



VIBRO - CONTROL
PIPETTE SHAKERS

Vibra-Control Shakers for blood pipettes are designed to produce even dispersion of the blood cells in the diluting fluid and without mutilation. Two sizes are available—one for holding two pipettes, and the other for six. Both shakers operate on 115 volt, 60 cycle, A.C.

No. 40310A Pipette Shaker for two pipettes	$17.50
No. 40310B Pipette Shaker for six pipettes	$42.50

NOTE THESE
FEATURES

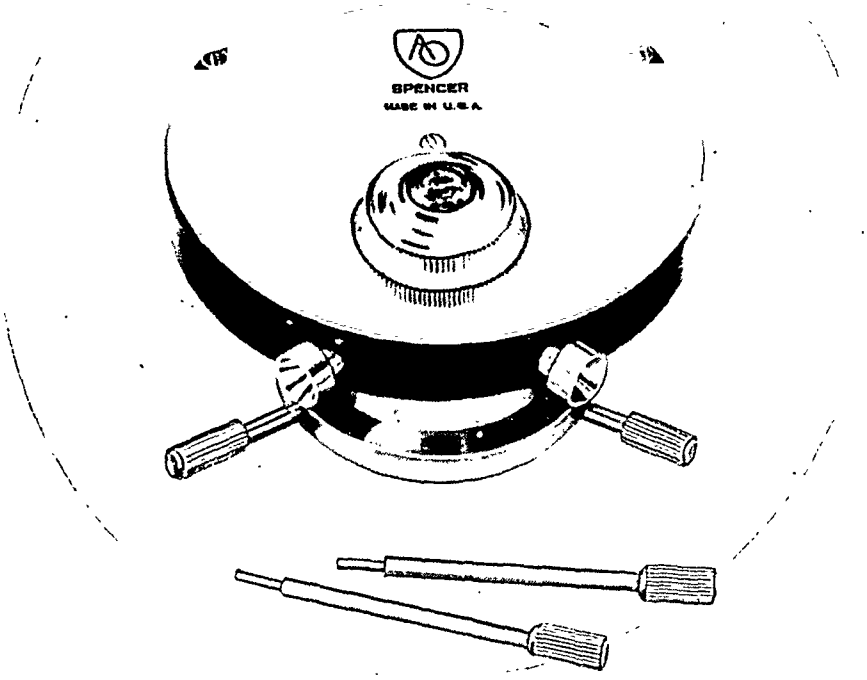
1. Combines rocking movement with gentle rotation.
2. Disperses cells evenly without mutilation.
3. Rubber cradle reduces breakage and holds all sizes of blood pipettes.
4. Voltage control permits adjustment to the correct rate of shaking
5. Attractively finished in white baked enamel for ease in cleaning.
6. Convenient to use

CENCO
CENTRAL SCIENTIFIC COMPANY
Scientific Apparatus
1700 IRVING PARK ROAD, CHICAGO 13
NEW YORK BOSTON SAN FRANCISCO NEWARK LOS ANGELES TORONTO MONTREAL

(In writing to advertisers, please mention the Journal—it helps.)

For EASE OF OPERATION
For PRECISION
For VERSATILITY

IT'S THE SPENCER
PHASE TURRET CONDENSER



TURRET DIAL ROTATES EASILY without danger of disturbing the microscope.
THE ANNULAR DIAPHRAGMS (interchangeable) are brought into correct position without backlash.
INDIVIDUAL CENTERING OF ANNULAR DIAPHRAGMS permits parcentering to four different phase objectives. Therefore there is no need to center annular diaphragms when changing objectives.
CENTERABLE MOUNT permits accurate location of phase condenser in substage fork.
LONG FOCUS ANNULAR DIAPHRAGMS to fit turret condenser are available for micro manipulation and tissue cultures located up to 17mm above stage.

Please write for demonstration or catalog and chart of applications.

Dept. Y 16.

American Optical Company
Scientific Instrument Division
Buffalo 15, New York

For Leadership in Phase Microscopy the World Looks to

SPENCER

(In writing to advertisers, please mention the Journal—it helps.)

Ortho anti Rh

These are the factors which govern the efficiency and usefulness of Anti-Rh Sera. Ortho Anti-Rh Sera, because they are prepared by skilled technicians from carefully selected human bloods are accurate, potent, avid, and stable.

Anti-Human Serum for Coombs "Developing" Test

Anti-Human Serum detects the presence of even minute amounts of blocking antibodies, thereby offering a wide field of usefulness in detecting "incomplete" Rh- to [illegible] during the gestation.

Packaging:
2cc. vials with a standardized dropper.

Available:

1. Anti-Rh_0 (Anti-D) Typing Serum for the Slide Test
 5 cc. vials (Approximately 200 tests)
2. Anti-Rh_0 (Anti-D) Typing Serum for the Test Tube Test
 2 cc. vials (Approximately 75 tests)
3. Anti-Rh_0^w (Anti-CD) 37% Typing Serum for the Test Tube Test
 2 cc. vials
4. Anti-rh' (Anti-C) Typing Serum for the Test Tube Test
 2 cc. vials
5. Anti-rh'' (Anti-E) Typing Serum for the Test Tube Test
 2 cc. vials
6. Anti-Human Serum for the Coombs "Developing" Test
 2 cc. vials

Anti-Human Serum is useful in:

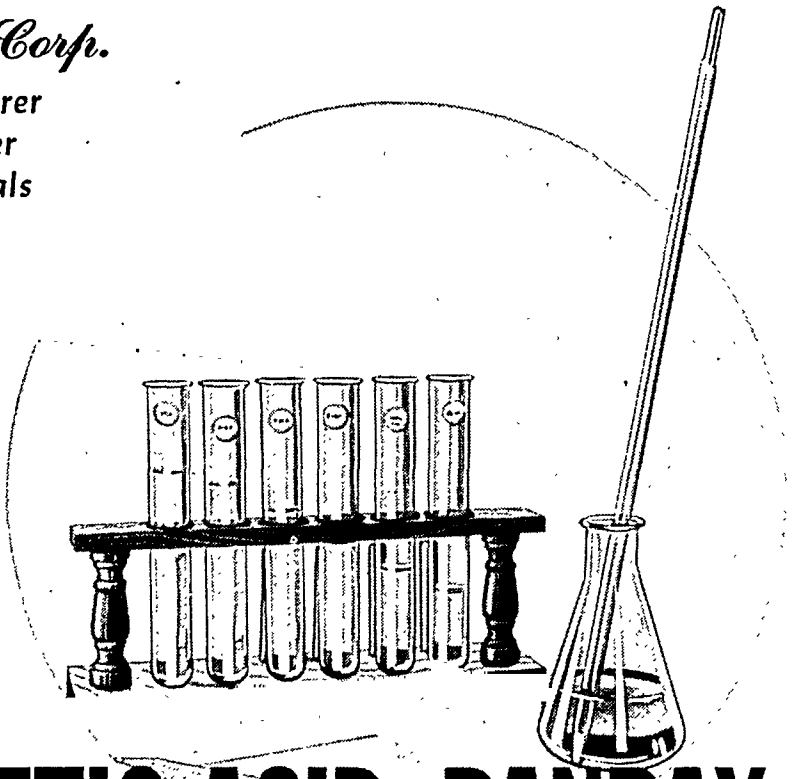
1. Detecting maternal Rh antibodies in cord blood or infants.
2. Checking the bloods of patients suspected of being immunized against the Rh or other factors.
3. Detection of those antibodies which fail to agglutinate saline suspended donor cells as in cross matching for transfusion.
4. Study and diagnosis of certain acquired hemolytic anemias.

Ortho

ORTHO PHARMACEUTICAL CORP., RARITAN, N. J.

(In writing to advertisers, please mention the Journal—it helps.)

The Panray Corp.
pioneer manufacturer
of DOPA and other
specialized chemicals
for investigation
and research...



announces the
availability of...

IODOACETIC ACID, PANRAY

TWICE RECRYSTALLIZED, SPECIAL REAGENT GRADE

for the Iodoacetate Index Determination of Serum Proteins

DOPA "PANRAY"
(3,4-dihydroxyphenylalanine, C. P.)
available in l and dl forms

Synthetic chemical reagents ... capable of revealing the presence of an oxidase in cells elaborating melanin. Valuable indicators of active melanogenesis.

Iodoacetic acid is a recently reported reagent for the determination of *deficient thermal coagulation of serum proteins* in cancer and other pathological conditions.

Originally reported by C. Huggins, M.D., G. M. Miller, M.D., and E. V. Jensen, Ph.D.: Thermal Coagulation of Serum Proteins—II. Deficient Coagulation in Cancer and the Iodoacetate Index—*Cancer Research, Vol. 9, No. 3, March 1949.*

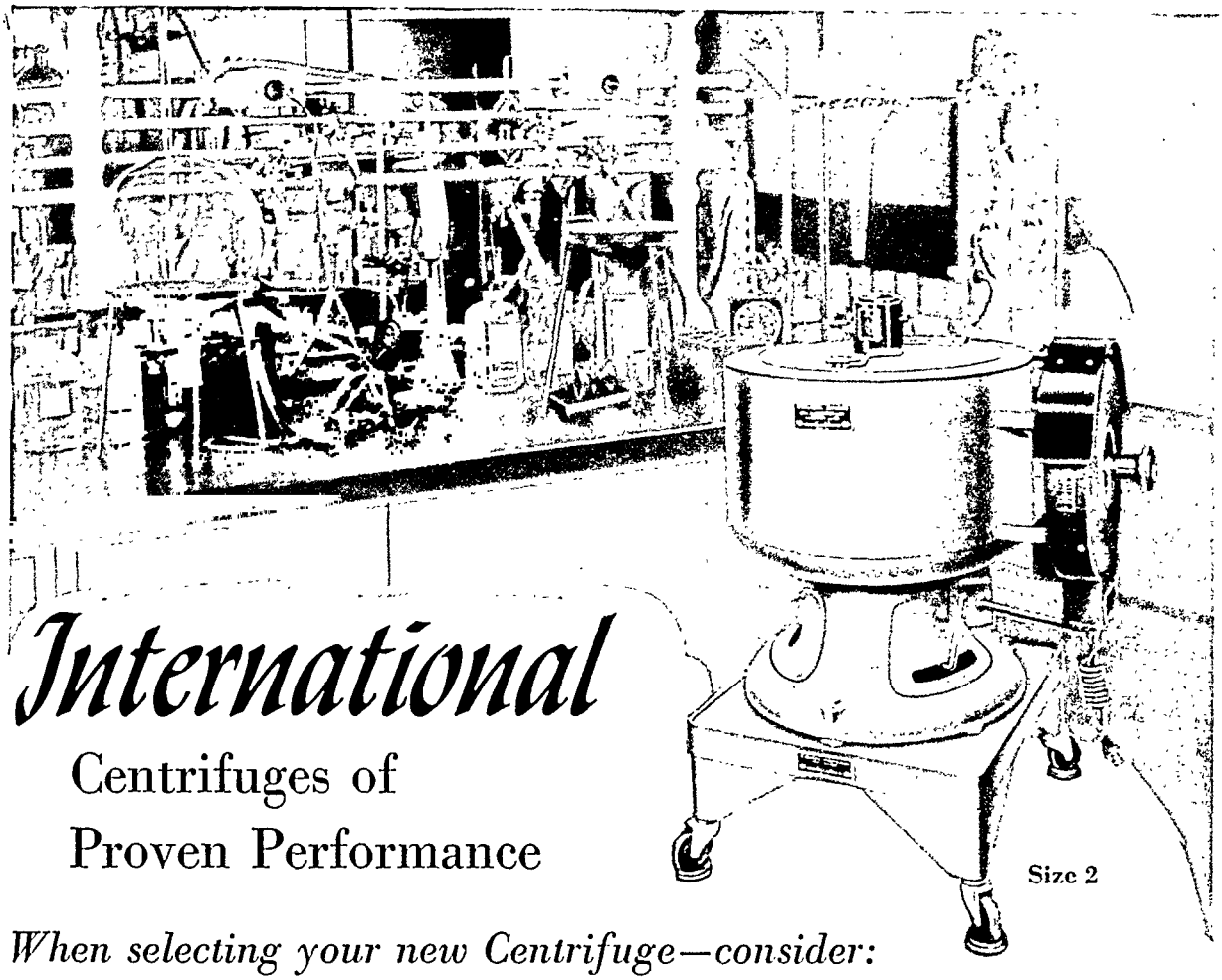
Write for prices and other pertinent data

The Panray Corp.

340 CANAL ST.
NEW YORK 13, N. Y.

Custom Manufacturers of Fine Organic Chemicals

(In writing to advertisers, please mention the Journal—it helps.)

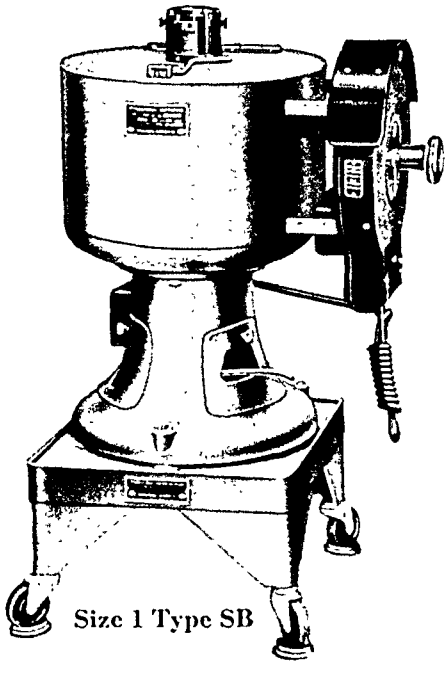


International

Centrifuges of Proven Performance

Size 2

When selecting your new Centrifuge—consider:



Size 1 Type SB

1 **The reliability of the electric motor that drives it.**
The motors of International Size 1 and Size 2 Centrifuges are specially designed to provide the best possible drive for a Laboratory Centrifuge. They are manufactured entirely in our own shops, thus eliminating the possibility of obsolescence through discontinuance by an "outside" manufacturer.

2 **The range of its application.**
Interchangeable accessories designed specifically for International Centrifuges are available in wide variety, making the machines adaptable to practically every laboratory application. Your future requirements as well as your present needs should be considered.

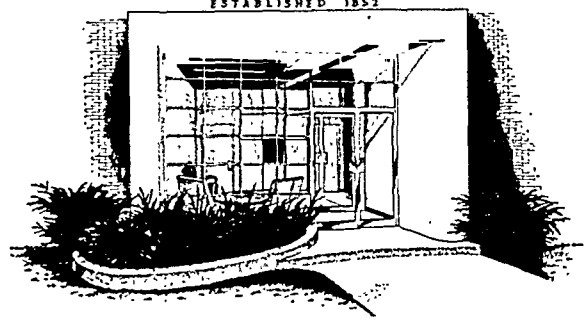
3 **The relation of first cost to total cost.**
Expensive repairs can make a low first cost a high cost in the long run. International Centrifuges are famous for their ability to give years of trouble-free service.

4 **The reputation of the manufacturer.**
International's fifty years of specialization in the manufacture of Laboratory Centrifuges is your guarantee of unequalled performance.

INTERNATIONAL EQUIPMENT COMPANY

1284 SOLDIERS FIELD ROAD, BOSTON 35, MASS.

E. H. SARGENT & CO.
ESTABLISHED 1852



Announcing **A NEW HOME**

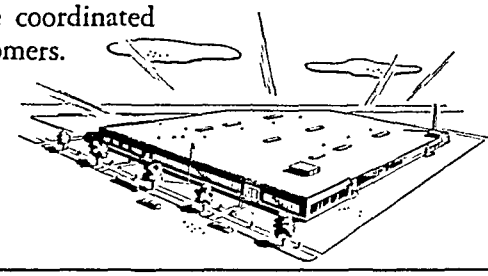
FOR A 97 YEAR OLD COMPANY

E. H. SARGENT & CO.

The three acres of floor space in this modern plant are designed for the ultimate in service to users of scientific laboratory instruments, apparatus and chemicals.

The modern new building houses complete engineering, production, merchandise handling and warehouse facilities—including a complete instrument factory; glass blowing shop with both grinding and graduating facilities; physical, chemical and preparation laboratories; complete engineering and drafting offices; all-steel merchandise storage structures with fire-proof storage rooms for hazardous chemicals; inspection department and packing room equipped with the latest type conveyor and inter-communication systems. Shipping facilities are a rail loading dock, rail spur and inside motor truck loading platforms.

Adjacent to the air conditioned general offices are located the city sales room, lecture and projection room, cafeteria with kitchen, scientific library and display room. All facilities are coordinated for expanded service to our customers. You are invited to visit us when in Chicago.



SARGENT

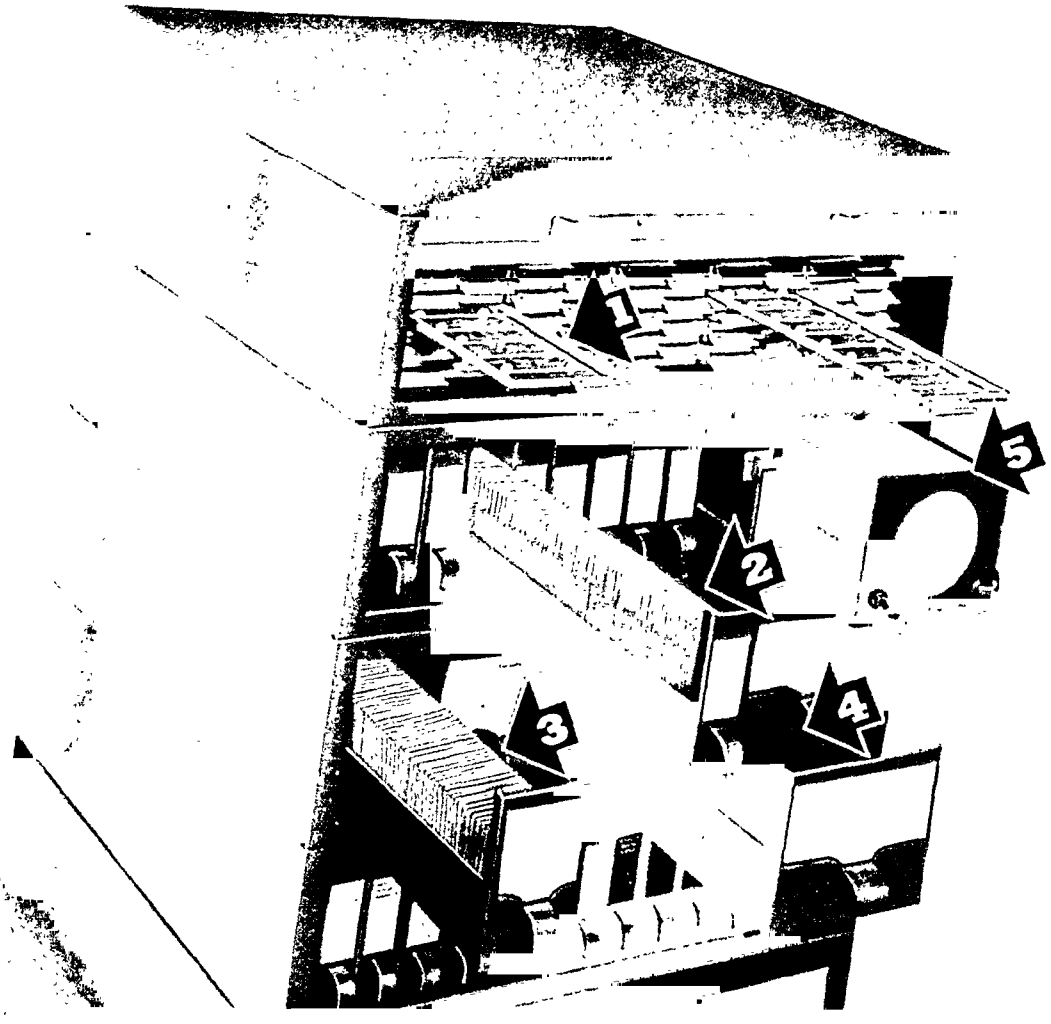
SCIENTIFIC LABORATORY INSTRUMENTS · APPARATUS · CHEMICALS

E. H. SARGENT & COMPANY, 4647 W. FOSTER AVE., CHICAGO 30, ILLINOIS

MICHIGAN DIVISION, 1959 EAST JEFFERSON STREET, DETROIT 7, MICHIGAN

SOUTHWESTERN DIVISION, 5915 PEELER STREET, DALLAS 9, TEXAS

(In writing to advertisers, please mention the Journal—it helps.)



ALL THIS (or other combinations)
in 19" square floor or desk space

1. Compartment-trays for drying and flat-filing microslides.
2. One-inch drawers for filing microslides vertically, either spaced or close-packed.
3. Two-inch drawers for Kodachromes and similar transparencies.
4. Four-inch drawer for lantern slides, index cards, or similar.
5. Four-inch drawer fitted with the TECHNILUME, a built-in spot illuminator for slide identification.

Lab-aid **the compact, flexible, all-steel laboratory filing system**

All vertical-filing drawers are interchangeable to make any combination your particular needs require. Bulletin No. 1600 describes this unique filing system. Please ask for it.

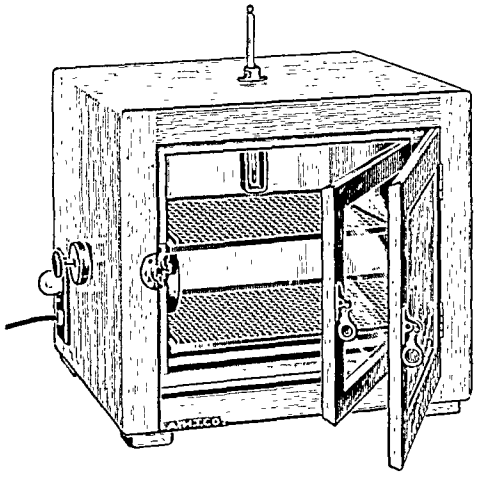
THE TECHNICON COMPANY • 215 East 149 St., New York 51, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)

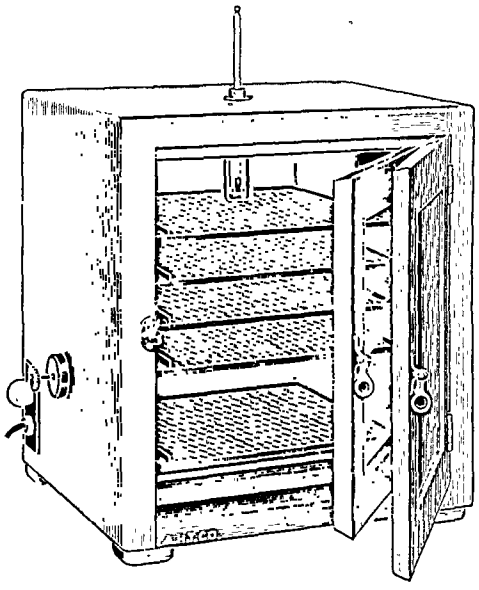
LIDBERG

PARAFFIN EMBEDDING OVENS

AGAIN AVAILABLE FOR IMMEDIATE SHIPMENT



1784.



1785.

PARAFFIN EMBEDDING OVENS, Lidberg Electric. Made of 5-ply laminated combgrain white oak, transite and hair felt insulation, with tinned copper lining throughout the embedding chamber. The heating unit consists of a unique woven wire mesh, embedded in asbestos, between the copper compartment and the transite wall on both sides and bottom. Temperature is controlled by a hermetically sealed expanding capsule thermoregulator with a temperature range from 50°C to 60°C and a sensitivity of 0.5°C. Ovens are adjusted for operation at 56°C, the attainment of which temperature requires approximately one hour and fifteen minutes from average room temperature.

The thermoregulator operates equally well on either alternating or direct current. Each oven is provided with an inner glass door and a drain hole in the center of the bottom.

Danger of fire from ignition of the volatile vapors from melting paraffin is reduced by the following features:

The contact points are hermetically sealed. Embedding chamber is lined throughout with tinned copper to prevent seepage of paraffin into contact with the heating units.

Heat of low intensity is uniformly distributed and not concentrated in localized spots of high temperature.

Operating sensitivity, i.e. the maximum variation of temperature at location of thermometer bulb, is ±1.0°C, when working within the usual range.

Operating uniformity, i.e. the maximum variation of temperature throughout the working shelf space in relation to the temperature indicated by the thermometer in the interior compartment, is ±2.0°C, under the same conditions.

1784. **Paraffin Embedding Oven, Lidberg, Electric,** as above described, inside dimensions 14 inches wide x 12 inches deep x 12 inches high. Minimum width of working space on lower shelf, 12 inches. With two shelves, 9603-B Thermometer and thermometer guard and complete operating directions. On 1 inch wooden feet for use on table. For 115 volts a.c. or d.c. 180.50
Net weight . 45 lbs. Shipping weight.....90 lbs. Code Word..... *Anode*

1785. **Ditto,** but with inside dimensions 15½ inches wide x 15 inches deep x 17½ inches high and with five shelves. For 115 volts a.c. or d.c. 256.25
Net weight ... 125 lbs. Shipping weight.....197 lbs. Code Word..... *Anofa*

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

Cable Address, "BALANCE", Philadelphia

NOT FOR OURSELVES ALONE*

PRESIDENTIAL ADDRESS

OSBORNE ALLEN BRINES, M.D.

From the Department of Pathology, Wayne University College of Medicine, Detroit 26, Michigan

In preparing this address, my first desire was to choose a worthwhile subject, one somewhat academic in scope or one dealing with the progress of medical science. Current impressions and influences, however, altered my intention as the title might indicate. Two years ago my concept of the activities of this society and the problems concerning the practice of pathology was probably similar to that held by most of you. Unless the vital questions and difficulties surrounding and attempting to engulf our rapidly developing specialty are beamed and concentrated in your direction, you are neither fully informed nor sensitive of great responsibility concerning them. To be forced to develop this state of awareness is the chief compensation of an officer of this society.

The American Society of Clinical Pathologists is blessed with a variegated membership composed of men and women in all phases and subdivisions of our specialty. As the name implies, this is an organization of clinical pathologists. Among its members are many teachers of pathology, experimental pathologists and others, all of whom are the friends and colleagues of the clinical pathologist; they understand his problems, sympathize with his viewpoints and supply many of his practical and intellectual needs. There are many like myself who are not immersed as deeply as some in clinical pathology but who by daily contacts and responsibilities possess an objective understanding and knowledge of the history and progress of this specialty. Perhaps this certain detachment, coupled with constant nearness, provides a greater interest and concern than may be developed by those who are more completely submerged. This does not constitute an argument, however, for failing to keep the security of this society in the hands of clinical pathologists, but suggests that those with somewhat diverse interests can be helpful in the future as they have been in the past.

It has been suggested to me several times that it would be helpful to everyone, including pathologists, to publicize a concise definition of clinical pathology or, perhaps, simply pathology. I believe the inference was that the recipients of this bit of educational propaganda should be the general public, the medical profession at large, our friends and families, our colleagues and co-workers—and ourselves. I will not go to the elementary extreme of proposing a formal definition, nor will I attempt to discuss and enlarge upon the obvious, but perhaps wordless, concept which has been accepted generally by all of you.

Clinical pathology and pathologic anatomy are divisions of general pathology.

* Presented at the Twenty-Eighth Annual Meeting of The American Society of Clinical Pathologists, in Chicago, October 12, 1949.

While a pathologist may place emphasis on, or restrict himself to, one subdivision or another, it is unwise for him to ignore the truth of that statement. Failure to do so has led many times to disinterest in and neglect of one branch or the other. Often when clinical pathology has been ignored, members of other specialties or technicians have stepped in to fill an unwarranted need. Some pathologic anatomists, interested in autopsies only, have practiced such a poor grade of surgical pathology that in some teaching hospitals general surgeons and surgical specialists are now practicing pathology.

Clinical pathology is a more diverse field than pathologic anatomy and is becoming so complex that it is difficult to be expert in all its phases. It is a specialty which readily provides specific interests. In fact, professional reputations are often made in one of the numerous "specialties within a specialty" which offer many opportunities for both investigation and application. Despite great possibilies in the practice and study of pathology, there is a tendency for some pathologists to complain and to look enviously at other specialties; while bemoaning their restricted opportunities, attractive fields of expanding interest are beckoning. I will mention a few examples. Medical biochemistry is an important field, scarcely explored by the average pathologist; it is easy to see now that we have left this domain too much to others. Microbiology, including virology and mycology, are not commanding the proper amount of the pathologist's attention. The average pathologist is not grealy interested in bacteriology and this science is not well developed in the average hospital laboratory. Modern hematology is so logically within the practice of pathology that it is somewhat shocking to observe how many hematologists and immuno-hematologists are in other specialties. Lack of interest on the part of pathologists in the operation of blood banks has created a deplorable and embarrassing national situation; it seems quite obvious that nothing is more squarely within the structure of the practice of pathology. The practice of toxicology by pathologists has become nearly extinct. In most communities today toxicological examinations are made only by law enforcement agencies or by industry; facilities are not readily available to study a case of poisoning in clinical or in necropsy material. In the field of laboratory criminology, a term which some of you may deplore, the knowledge and the wisdom of pathologists is acutely needed. The reluctance of the average pathologist to enter the field of medical jurisprudence has done much to delay the abolishment of the archaic coroner's office and the general adoption of the modern medical examiner system with facilities for performance of proper and adequate medicolegal autopsies. Early aloofness on the part of some pathologists diverted exfoliative cytology to other specialists in some instances. It probably will not be long before chemical or serologic tests for cancer will be performed routinely; if so, I hope they will be included within the realm of clinical pathology and they will be if pathologists are sufficiently interested, aggressive and willing to explore and develop this new field. The list could be extended much further, at least to include medical biophysics which medical schools have not yet seen fit to emphasize.

Conditions under which the pathologist has been forced to practice his specialty

are partially responsible for the state of affairs to which I have just alluded. Undesirable hospital conditions, including the old master-servant relationship, have been somewhat to blame. The pathologist individually has not been able to do much about such situations, but pathologists collectively can. Many pathologists are expected to operate a large department with insufficient professional assistance. The hospital administrator may grudgingly allow him a resident or two, whereas he may actually need one or two assistant pathologists as well as residents. Such lack of personnel often explains his discouragement and inability to expand to collateral fields in which he is competent and which he is anxious to explore and develop. He is often restricted to the direction of a suppressed department which requires his entire attention; under such adverse conditions he is not given a fair chance to develop his full professional stature.

Where are young pathologists to be found to staff these properly expanded hospital laboratories? The answer is probably not a concise or an immediate one, but it involves interesting better medical students and internes in the practice of pathology. I specify "the better" because this specialty no longer attracts merely those who are doubtful of their ability to practice clinical medicine successfully. Before interesting and convincing others it is necessary for us to become more familiar with our own specialty—more conversant with its opportunities and advantages. Many pathologists have not contributed much to the recruitment of younger men into the field; some have actually advised adversely. Much has been accomplished but much more needs to be done to make this a more attractive and desirable specialty. The newer aspects and improved conditions of practice must be brought to the attention of those whom we desire to impress. There is a regrettable tendency for older and more successful pathologists, whose economic needs have long since leveled off or declined, to be complacent about situations which obviously need improving or correcting. The alert, enterprising and progressive pathologist will not find such selfishness a part of his philosophy. Nowhere could the words "not for ourselves alone" be more appropriate.

The meaning of pathologic anatomy, as difficult as it is at times to convey to the laity, is clear enough to the members of the medical profession; its companion specialty requires more concentrated thought. Clinical pathology is the practice of laboratory medicine. It consists of observing and measuring certain physical, physiological and chemical phenomena for the purpose of diagnosis or treatment and involves the correlation and application of results thus obtained. The pathologist may achieve such results by actual performance or by direction; the more intimately the two methods are combined, the more successful will be his practice. The complexity and magnitude of this specialty require the help of assistants who are usually medical technologists, our colleagues and members of an allied profession. At times our assistants and co-workers are Ph.D's in one of the sciences related to medicine. Unfriendliness and jealousy sometimes creep into this relationship and this is both unfortunate and unnecessary. We need the Ph.D's and should recognize them as our worthy collaborators. We should work in harmony with them and bring their work into focus with the practice of

medicine. It is quite obvious that the practice of clinical pathology will vary greatly depending on the type and size of the organization involved, whether it is the operation of a hospital or private laboratory, or some other type of pathological practice.

The practice of a specialty should consist not only of acquiring knowledge, information and data, but of applying them. The pathologist usually limits his contact with patients to a consultation practice but frequently this contact is quite remote, more or less limited to technical aspects. Often he does not see the patient and is content not to, but thereby he misses a great opportunity to develop his practice into a worthwhile consultation service. He usually has only to indicate his availability as a diagnostic consultant to have his opinion and services sought by his clinical confreres. Often his aloofness or unwillingness to serve in this capacity results in losing another fine opportunity to bring laboratory medicine to the bedside of the patient.

The separate components of clinical pathology, for example, clinical chemistry, bacteriology or one of its subdivisions, hematology, blood banking, cancer diagnosis, medical biophysics, or one of several other branches of applied science, individually or severally, may attract the interest of the pathologist and, as a rule, constitute his basic work pattern. Quite often, regardless of his routine practice, one of the above may become his special interest and study, and form the basis of his professional prestige. Frequently I have been suprised and slightly amused to find eminent "name" pathologists doing the same routine work in their own laboratories that the rest of us back home were doing in ours. The contribution which had made them outstanding had been only a part of their practice—sometimes an extra-curricular activity, almost a hobby. In no other specialty is the variety of interests so wide and the possibility of their development so great.

We have all been told repeatedly that the laboratory is the educational heart of the hospital. Sometimes this was flattery, preparatory to handing us the chairmanship of the library committee or asking us to arrange a series of lectures for the internes. Nevertheless, the pathologist has an educational responsibility which he must discharge, generally, in the direction of the attending, resident and interne staffs; specifically, for his own residents and students in medical technology. His medical school affiliations may be such as to provide him a satisfactory opportunity for more formal teaching, but in any event, one of the pathologist's most important roles is that of teacher.

No other specialty provides such a natural opportunity for clinical and laboratory investigation. The pathologist is in a most favorable environment and possesses the basic facilities for independent research and the opportunity to collaborate with others. Many pathologists say that they are too busy or that they are "not the research type". I fear many of us have been guilty of such an attitude from time to time, but let us be honest enough to admit that we were just lazy. The pathologist of the future with his scientific training and experience will not be able to exist happily—to breathe professionally and scientifically—unless engaged in some phase of medical research.

The pathologist is not a delicate creature who must not be disturbed in his sanctified surroundings or removed from his rarefied atmosphere and exposed to the bright sunlight of reality. He is a rugged individual who is an active member of his local medical profession; he should be active in civic affairs; he should have a wide acquaintanceship both within and outside his profession; he should be a potent force in his hospital staff organization; he is the administrator of an important department and he should be interested and well informed about the administration of other departments and of the hospital as a whole. His influence should be felt in shaping the professional policy of the hospital. He may find that much of his time is consumed by various committees of which he is a member and that some of these activities are irksome, but these are duties and opportunities which he must not shirk.

Starting with the intention of proposing a definition of pathology, I have indulged in a lengthy dissertation of what constitutes and what may be included in this specialty. The latter was perhaps the easier.

This is not a clinic for the diagnosis of the ills of the practice of pathology. Never have our ideas regarding existing defects and desired improvements been so clear and so well oriented. The ills have always been there, but now we are more literate and more articulate. Today we have media of expression in the form of democratic societies through which we may make ourselves heard and felt. We are now professionally quite healthy and should give more thought and energy to self-perpetuation. That young pathologists are not being produced in necessary quantities is somewhat a reflection on ourselves. This is not a specialty which has neophytes beating on its doors. The recruitment of men and women into this specialty will require education and publicity in the field of professional and public relations. It will require missionary zeal and aggressiveness to convert young prospects to take this better way of scientific life. Perhaps "by example" is the best way, being willing to let the good deeds shine forth. What I seriously have in mind is the tendency of many pathologists to work in semi-seclusion, secretly and mysteriously, thereby preventing the potentially interested medical student or intern from obtaining a comprehensive understanding of the practice of pathology.

Perhaps it has been quite inappropriate and unnecessary to interrupt the scientific program in this manner to discuss the state of our specialty and our practice. However, brief introspection mingled with a justifiable expression of pride in our accomplishments, even though not long remembered or deeply ingrained, may clarify our concepts and stimulate us to renew our endeavors.

THE INCIDENCE OF POSTTRANSFUSION SERUM HEPATITIS*

JOHN J. McGRAW, JR., M.D., MAX M. STRUMIA, M.D., AND ELIZABETH BURNS, R.N.

From the Laboratory of Clinical Pathology of the Bryn Mawr Hospital and the John S. Sharpe Research Foundation of the Bryn Mawr Hospital, Bryn Mawr, Pennsylvania

The purpose of this paper is to report the results of an investigation to determine the incidence of hepatitis in patients transfused with blood and/or plasma at the Bryn Mawr Hospital. Patients in the same general disease category, who were not transfused, were used as controls.

Serum hepatitis, or homologous serum jaundice, transmitted by the injection of blood or blood products, is a disease which has recently assumed considerable importance. The disease is not new but was brought to the attention of the medical profession with considerable force in 1942 when many soldiers developed jaundice after they had been inoculated with certain lots of yellow fever vaccine.[1] The vaccine contained human serum which was believed to harbor the etiologic virus. The subject has been excellently reviewed by Neefe.[5] Very small amounts of serum may transmit the disease and it is probable that a single donor may contaminate a large pool of plasma or serum. The virus is resistant to heat at 56 C. for at least 60 minutes and apparently is not destroyed by ordinary concentrations of Merthiolate (Eli Lilly and Co.) and certain other bacteriocidal agents. It is well preserved by drying and freezing but its stability in the liquid state at room temperature has not been well defined. Ultra-violet irradiation, properly controlled, destroys the virus without significant alteration of the plasma proteins.

The exact relationship of serum hepatitis to naturally acquired infectious hepatitis has not yet been established. Clinically the two diseases are much alike and pathologically they are almost identical. Immunologically there are certain differences[6] which are open to various interpretations. The incubation period of naturally occurring infectious hepatitis is approximately 20 to 40 days. The incubation period of infectious hepatitis transmitted by injection of a patient's serum to volunteers is usually short (less than 40 days), while the incubation period for serum hepatitis is usually 60 to 150 days. Most studies have shown that an attack of one disease confers some immunity to that disease but not to the other. At least one study, however, suggests that there may be some cross-immunity between some strains.[7]

SERUM HEPATITIS AT THE BRYN MAWR HOSPITAL

Bryn Mawr is a hospital in a suburb with a relatively stable population. Its blood and plasma service is operated by the Laboratory. Most of the donors are obtained from the local population but some are temporary residents of nearby colleges. All donors are subjected to the usual brief medical history and physical examination. A history of jaundice within two years is cause for rejec-

* Received for publication, June 27, 1949.

tion. The serums used for the serologic tests are screened and any which have a suspiciously yellow color are checked for bilirubin content. If the bilirubin level is over 1 mg. per 100 ml., the blood is discarded.

Blood is drawn into acid-citrate-dextrose solution and used for whole blood transfusion within 21 days. Blood not used within 21 days is converted to plasma. Approximately 3,500 donors are bled each year. From the blood obtained, approximately 1,500 units are used as whole blood and the remainder is converted to pooled plasma. The group and Rh-type of each blood is determined. The group O blood is titered to determine those which have a high content of anti-A or anti-B agglutinins. Group A, B, and AB patients are given low-titer group-O blood routinely. When the supply in the bank is low, group A patients may be given Group A blood during the regular laboratory hours. Rh-negative patients receive Rh-negative blood.

Pooled plasma is made from outdated group O bloods and from fresh group A, B and AB bloods. Each pool contains plasma from 8 to 12 donors. The great bulk of the plasma is stored in the frozen state and a small proportion is dried from the frozen state. No bacteriostatic or bacteriocidal agents are added to the plasma.

In view of the fact that complete records were available, it was considered advisable to investigate the incidence of hepatitis in the recipients of blood and plasma. In addition to plasma prepared at the Bryn Mawr Hospital, a small amount of surplus Red Cross plasma also was used. Questionnaires were sent to all patients receiving blood or plasma during the past four years, asking whether they had had jaundice within six months of their last transfusion. Letters and questionnaires were sent to 1,523 patients who had been transfused, and 936 satisfactory replies were received.

In addition to the cases of jaundice uncovered by the questionnaires jaundice was reported by the attending physician in 7 transfused patients; in most of these patients the jaundice was reported before the survey was inaugurated. These patients did not receive a questionnaire but are included among those giving a positive answer. Four of these 7 patients were listed with those having "probable hepatitis".

Jaundice in the control group. It was realized that it was not possible to assemble a completely satisfactory group of controls. It is only fair to assume that, within a single hospital, the patients who receive transfusions are more ill than those who do not. However, a reasonably satisfactory group of controls was selected from those patients who were hospitalized at the same time as the transfused group. They were selected from the listings in the hospital admission book. The number of controls selected for each month was the same as the number of patients transfused in that month. The ages, sex and grouping by hospital service were approximately the same.

The same form letter and questionnaire mailed to the transfused group were sent to representative controls and the first 1000 satisfactory answers were used.

Interpretation of replies. The negative answers were accepted without further investigation. Letters with affirmative answers were investigated as thoroughly

TABLE 1

SUMMARY OF DATA IN PATIENTS WHO STATED THAT THEY HAD JAUNDICE WITHIN THE SIX-MONTH PERIOD AFTER TRANSFUSION

(In this analysis the study period is considered to start two weeks after transfusion and extend for six months)

PATIENT			DISEASE FOR WHICH PATIENT WAS HOSPITALIZED	TRANSFUSIONS*		ONSET OF JAUNDICE		POSSIBLE SERUM HEPATITIS	PROBABLE SERUM HEPATITIS	REMARKS
No.	Age	Sex		Type	No.	During study period	Days after transfusion			
T 1	53	F	Common duct stone, jaundice	Pl B	4 1	No		No	No	Improvement after operation
T 2	55	F	Cholelithiasis, carcinoma stomach	Pl	7 1	No		No	No	Mild jaundice first week post-operative
T 3	47	F	Cholelithiasis, common duct stone, jaundice	B	1	No		No	No	Improvement after operation
T 4	35	F	Hypertrophied ligamentum flavum	B	1	No		No	No	Mild intestinal upset; never jaundiced
T 5	30	F	Ulcerative colitis	Pl B	4 1	No		No	No	Colectomy; mild jaundice first week postoperative
T 6	61	M	Cholelithiasis, jaundice	Pl B	5 7	No		No	No	Jaundice improved after operation,
T 7	38	F	Hemolytic jaundice	Pl B	2 4	No		No	No	Splenectomy; good results
T 8	47	F	Cholelithiasis	Pl B	2 1	No		No	No	Jaundice before admission
T 9	40	F	Hemolytic jaundice, anemia	B	12	No		No	No	Splenectomy; good results
T10	35	F	Acute infectious hepatitis	B	3	No		No	No	Admitted with hepatitis
T11	62	F	Acute cholecystitis with perforation	Pl B	1 1	Yes(?)		Yes	No	Some pruritis, no icterus one month after discharge
T12	40	M	Hemorrhoids; hemolytic jaundice?	Pl B	24 5	Yes(?)		Yes	No	Hemolytic anemia; bilirubin 1.6 mg. 95 days later
T13	41	F	Carcinoma pancreas, jaundice	Pl B	6 1	Yes	150	Yes	No	Jaundice recurred in five months; died of carcinoma
T14	45	F	Carcinoma pancreas	B	1	Yes	30	Yes	No	Jaundice also 30 days after transfusion, from carcinoma

T15	70	F	Acute cholecystitis	Pl B	1 1	Yes	130	Yes	No	Clin. diag., serum hepatitis; autopsy, common duct stone
T16	69	M	Mesenteric thrombosis	Pl B	8 1	Yes	63	Yes	No	Clinical, serum hepatitis; autopsy, severe portal cirrhosis
T17	73	M	Hemolytic anemia?	Pl B	8 1	Yes	100	Yes	No	Clinical diagnosis, serum hepatitis; autopsy, severe liver damage, lipogranuloma
T18	62	M	Duodenal ulcer, perforation into pancreas, cholelithiasis	Pl B	7 13	Yes	30	Yes	No	Postoperative intraperitoneal bleeding, jaundiced one week later
T19	68	M	Chronic cholecystitis, cholelithiasis, jaundice	Pl	1	Yes	35	Yes	Yes(?)	Jaundice before admission, lasted one month; R.
T20	68	M	Acute cholecystitis, cholangitis	Pl	1	Yes	65	Yes	Yes(?)	Cholecystectomy; biliary disease or virus hepatitis; R.
T21	31	F	Poliomyelitis	Pl	4	Yes	40	Yes	Yes	Probable serum hepatitis; R.
T22	56	M	Sigmoidal diverticulitis, abscess	B	3	Yes	40–54†	Yes	Yes	Abscess removed; probable serum hepatitis; R.
T23	49	F	Leiomyomata	RC Pl B	1 1	Yes	50	Yes	Yes	Probable serum hepatitis; R.
T24	6	F	Severe burns	Pl B	24 18	Yes	0–120†	Yes	Yes	Probable serum hepatitis; R.
T25	16	M	Poliomyelitis	Pl	2	Yes	90	Yes	Yes	Probable serum hepatitis; R.
T26	53	F	Fractured ribs, cerebral concussion	Pl	4	Yes	70	Yes	Yes	Probable serum hepatitis; R.
T27	23	M	Post partum hemorrhage	Pl B	2 1	Yes	60	Yes	Yes	Probable serum hepatitis; R.
T28	63	M	Peptic ulcer, gastrectomy	Pl B	17 1	Yes	30–150†	Yes	Yes	Probable serum hepatitis; R.
T29	58	M	Chronic glomerulonephritis, nephrotic stage	Pl	29	Yes	0–245†	Yes	Yes	Probable serum hepatitis; R.
T30	64	F	Chronic cholecystitis, cholelithiasis	Pl	3	Yes	120	Yes	Yes	Probable serum hepatitis; autopsy, severe acute hepatitis

* Pl, plasma; B, blood; RC Pl, Red Cross plasma; R., recovery.

† Lower figure represents number of days after last transfusion; higher figure, number of days after first transfusion.

as possible, the hospital records were reviewed, and contact was made with the attending physicians. Whenever this did not clarify the situation sufficiently, further details were obtained directly from the patient. Only those patients who were transfused, and who developed jaundice from two weeks to six months after the transfusion, were considered to have had jaundice which started during the study period. For the controls, only those who developed jaundice within six months after admission to the hospital were considered to have developed jaundice within the study period.

Patients who developed jaundice within the study period were further studied in an attempt to decide whether the jaundice was attributable to hepatitis or to other causes. All patients who developed jaundice in the study period were recognized as having "possible hepatitis" even though other associated lesions, such as common duct stones or carcinoma of the pancreas, seemed more likely to be the cause of the jaundice. As a result, the incidence of "possible hepatitis" in the transfused group is almost certainly higher than the incidence of true hepatitis.

All patients who developed jaundice in a six-month period starting two weeks after transfusion and whose signs and symptoms could not be definitely attributed to disease other than acute or subacute hepatitis were classified as "probable hepatitis". The figures are possibly higher than the actual incidence of true hepatitis. A certain number of cases which would have been listed as "probable hepatitis" were moved back into the group of "possible hepatitis" when subsequent postmortem examination revealed such lesions as long-standing portal cirrhosis or silent common duct stone. The incidence of "possible hepatitis" and "probable hepatitis" in the control and transfused groups is listed in Table 2. The transfused group of patients returned 30 affirmative answers. Ten of these were eliminated because the jaundice was present prior to transfusion or developed within a few days. The remaining 20 (2.1 per cent) were classified as having "possible hepatitis". In 8 of these, the jaundice almost certainly was not related to the transfusion. This left 12 patients (1.3 per cent) who were classified as having "probable hepatitis" following tranfusions of blood and/or plasma. The control group contained only 2 persons (0.2 per cent) with "probable hepatitis".

Blood vs. blood and/or plasma. Inasmuch as Bryn Mawr Hospital pooled plasma contains plasma from 8 to 12 donors, it might be expected that the incidence of serum hepatitis would be greater from pooled plasma than from blood alone. The transfused group was studied in an attempt to analyze the findings on this basis. Of the 936 answers received, 382 were from patients who received blood alone, and 528 received plasma alone or blood and plasma. The group of patients who received blood alone contained two persons (0.5 per cent) with "possible hepatitis" and one (0.26 per cent) with "probable hepatitis" in the study period. Among the group of persons who received plasma alone or blood and plasma, 18 persons (3.4 per cent) had "possible hepatitis" and 11 (2.1 per cent) "probable hepatitis" (Table 2).

It might be anticipated that pooled plasma would be followed by a higher in-

cidence of serum hepatitis than whole blood. If a pool contains plasma from 10 donors there is 10 times the chance that it may be contaminated by the virus. In addition, it is possible that the pool may be divided into 10 units and that each unit may be given to a different patient. In actual practice, however, a single patient often receives the bulk of one pool. There are additional factors which probably tend to increase the incidence of serum hepatitis in patients who receive transfusions of plasma. In our hospital, most patients who receive plasma are given several units over a period of time. This is true to a far greater extent for plasma than it is for whole blood. The rate of multiple transfusions was determined for the 936 patients studied in this survey; of 604 patients who received blood, only 50 per cent received more than one unit of blood; of 512 patients who received plasma, almost 85 per cent were given more than one unit.

Incidence of hepatitis per transfusion. In the paragraphs above, the incidence of hepatitis has been determined by comparing the number of cases of hepatitis

TABLE 2

INCIDENCE OF HEPATITIS FOLLOWING VARIOUS TRANSFUSION FLUIDS

TRANSFUSION FLUID	NUMBER OF PATIENTS	NUMBER AND PER CENT WITH POSSIBLE HEPATITIS	NUMBER AND PER CENT WITH PROBABLE HEPATITIS
None (controls)	1000	2 (0.2%)	2 (0.2%)
Whole blood only	382	2 (0.5%)	1 (0.3%)
Whole blood and plasma	249* } 528	11 (4.4%) } 18 (3.4%)	4 (1.6%)‡ } 2.1%
Plasma only	279† }	7 (2.5%) }	7 (2.5%) }
Globin only	26	None	None

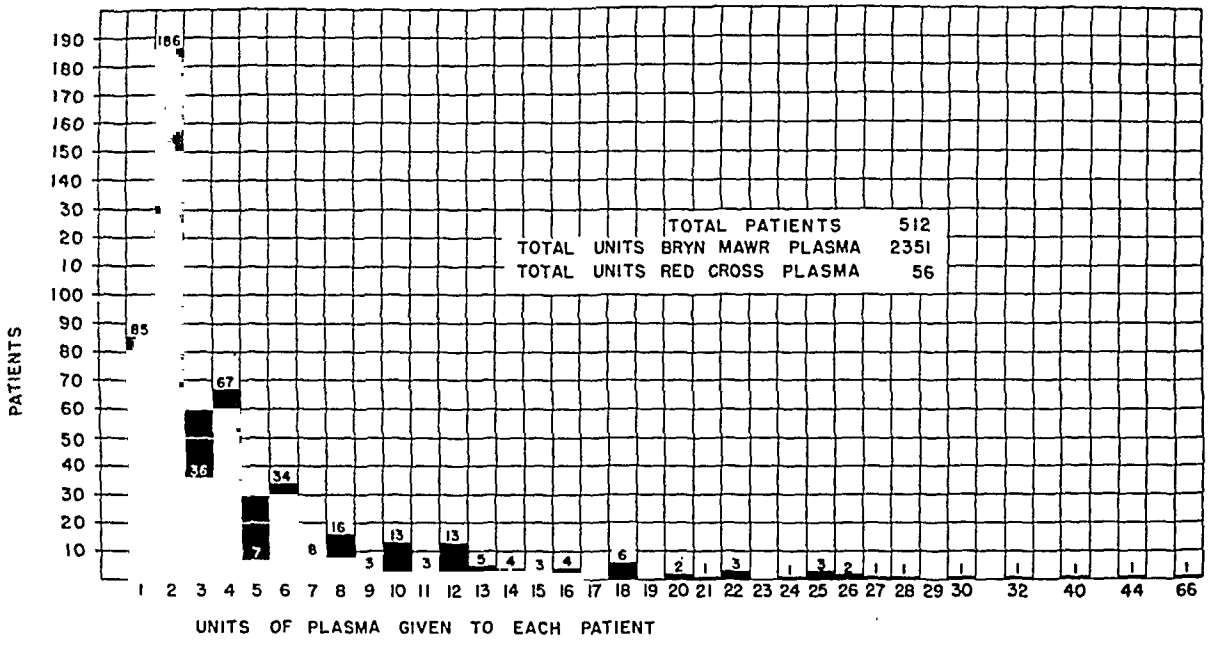
* 27 of these received surplus Red Cross plasma in addition to Bryn Mawr plasma.

† Plasma prepared at the Bryn Mawr Hospital.

‡ The plasma received by one of these patients was Red Cross surplus.

with the number of transfused patients without regard for the number of transfusions given. Thus our incidence of 1.3 per cent of hepatitis in 936 patients transfused with blood or plasma means that 1 out of 77 of these patients developed hepatitis which was probably transmitted by the tranfusion. It does not mean that 1 out of 77 transfusions resulted in hepatitis. Most of the patients received multiple units of blood and/or plasma and, consequently, the incidence of disease per transfusion is correspondingly lower.

In the period of four years covered by our survey, 4,139 blood transfusions and 8,256 plasma transfusions were given at this hospital. Thus we were able to find only 12 cases of probable serum hepatitis during a period in which 12,395 transfusions were administered. This is almost certainly a misleading figure although estimates derived in such a manner have been presented in at least two reports. A more accurate estimate of the incidence of hepatitis according to the number of transfusions may be obtained by totaling the number of units of blood and plasma given to the 936 patients who answered our questionnaire. Taken as a group these patients received 3,723 units of blood (500 cc. per unit) or plasma (250 cc. per unit). The 12 cases of hepatitis in this group give an incidence


PATIENTS
TOTAL PATIENTS 512
TOTAL UNITS BRYN MAWR PLASMA 2351
TOTAL UNITS RED CROSS PLASMA 56
UNITS OF PLASMA GIVEN TO EACH PATIENT

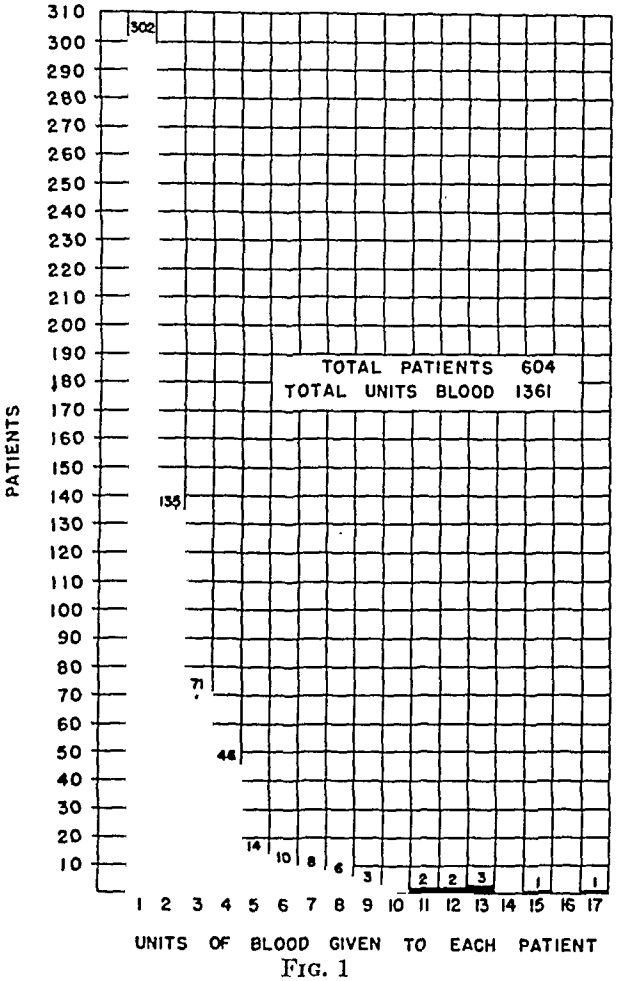

PATIENTS
TOTAL PATIENTS 604
TOTAL UNITS BLOOD 1361
UNITS OF BLOOD GIVEN TO EACH PATIENT

FIG. 1

of 12 per 3,723 or approximately 1 per 300 transfusions of blood and/or plasma. One of the 12 patients received whole blood alone and another received Army surplus plasma distributed by the Red Cross. The remaining 10 received Bryn Mawr plasma and the number of units of such plasma given to the group of patients studied totaled 2,351. Thus the incidence of hepatitis per unit of Bryn Mawr plasma was 10 per 2,351 or approximately 1 per 235.

Figure 1 shows the number of transfusions of blood and plasma given to the 936 patients who answered the questionnaire. The figures are broken down to indicate the number of patients who received multiple transfusions and the number of units given to these persons. It is interesting to note that, out of 509 patients who received plasma, 424 received more than one unit and the average amount of plasma given to each patient was over 4 units. By comparison, only 302 of 604 patients received more than one unit of whole blood. The average amount of blood given to transfused patients was just over 2 units.

Investigation of possible contamination of plasma pools. Although some authors[10] have incriminated single pools as causing jaundice in many patients, in this study only two patients with "probable hepatitis" received blood from the same two pools. It was impossible to determine which donors gave contaminated blood, in that some patients received blood from many pools.

DISCUSSION

It may be argued that the control series was not adequate and that the patients in the transfused groups were more ill and, hence, more susceptible to naturally occurring infectious hepatitis or to hepatitis from any other cause. There is probably some merit to this argument and it is possible that some of the cases of hepatitis following transfusion were coincidental. There is no way of differentiating between serum hepatitis and naturally occurring hepatitis except by transmission-experiments with human volunteers. The figures obtained from the present clinical survey cannot have the absolute controls of a planned laboratory experiment. It is necessary to accept this limitation and to pass judgment accordingly. It is our opinion that the difference in the incidence of hepatitis in the controls and in the transfused groups cannot be explained by the lowered resistance of the transfused patients to naturally occurring disease alone. There were no other cases of hepatitis in the patients' families at the time and we believe that most of the cases labeled "probable hepatitis" in the transfused group were transmitted by transfusion.

The fact that we have used the term "probable hepatitis" may suggest that a certain number of the cases were not true hepatitis. This argument, too, has merit. Thus in each of Cases 15, 16, and 17, the attending physician made a tentative diagnosis of serum hepatitis which was later proven to be erroneous by postmortem examination. Only in Case 30 were we able definitely to establish the diagnosis of hepatitis histologically. It may be pointed out that in our operation of the blood and plasma service at this hospital, we tended to criticize

FIG. 1. Charts indicating number of transfusions given to 936 patients who answered the questionnaires, number of persons who received multiple transfusions and number of units of blood and plasma administered.

ourselves more severely than a less interested observer might. Thus in Cases 19 and 20 there was previous gallbladder disease but the recurrence of jaundice was attributed to "probable hepatitis" because it fitted the clinical picture and occurred within the accepted incubation period. However, in Cases 21 to 30, inclusive, there is no doubt that the jaundice was due to hepatocellular disease. Unlike many other surveys of this type we had close liaison with the attending physicians and personal knowledge of the history, physical findings and laboratory tests of most of the patients. It is our opinion that the cases listed as "probable hepatitis" are true examples of hepatitis. We used the adjective "probable" since we consider histologic examination to be essential for an absolute diagnosis.

It is interesting to compare our findings with those reported in other surveys. Before we do this, it must be pointed out that there are many variable factors which limit the conclusions that may be drawn from such comparisons. In some instances the pools are very much larger than others. In many surveys no attempt was made to establish a control series for comparison with the transfused groups. In almost all, there is no accurate estimate of the incidence of infectious hepatitis in the local area at the time the survey was made. This is almost certainly an important factor both from the standpoint of infected donors and from the standpoint of naturally occurring disease in the recipients. Since infectious hepatitis is not often reported to public health agencies, this information is not easily obtained. We believe that the low incidence of hepatitis in the nontransfused controls indicates that relatively few of the cases of hepatitis in the transfused group were naturally acquired. Finally, there are great variations in the methods used for calculating the incidence of hepatitis. Some of these vary so much that it is not valid to compare the results of different reports. For example, Spurling, Shone and Vaughan,[10] and Brightman and Karns,[3] reported incidences of hepatitis of 7.3 per cent and 4.5 per cent, respectively, based upon the number of transfused patients without regard for the number of transfusions given; whereas Grossman and Saward,[4] and Scheinberg, Kinney and Janeway[9] determined incidences of hepatitis of 1.6 per cent and 1.1 per cent, respectively, according to the number of plasma transfusions without regard for the number of patients transfused. Consequently, the figures of the former two groups of workers are not at all comparable to those of the latter two groups. Determination of the incidence of hepatitis based on the number of transfusions given in any period is certain to result in a lower incidence than figures based upon the number of patients transfused because many patients receive multiple units of blood or plasma. In order to compare our results with those of other surveys, the incidence of hepatitis at the Bryn Mawr Hospital was determined both according to the number of transfusions and according to the number of patients transfused.

Two and one-tenth (2.1) per cent of our patients who received plasma developed hepatitis probably as a result of the transfusions. This figure is comparable to those reported by Brightman and Karns (4.5 per cent) and by Spurling *et al.* (7.3 per cent). Although it is not possible to account for all of the variables mentioned above, it is tempting to explain the differences, at least in part, on the basis of the size of the plasma pools. Our pools were relatively small (8 to 12

donors). The pools which showed a 4.5 per cent incidence were from the Army surplus distributed by the American Red Cross and had a larger number of units (25 to 50). The British pools which gave a 7.3 per cent incidence of hepatitis were still larger, having plasma from as many as 800 donors. The incidence of hepatitis, at least in these three surveys, increases with the increased size of the pools.

Our incidence of hepatitis of 0.4 per cent (1 case per 235 plasma transfusions) may be compared with 1.6 per cent (8 in 501 transfusions) reported by Grossman and Saward and 1.1 per cent (1 per 86) reported by Scheinberg *et al.* Our figure which is considerably lower might be explained in part by the fact that, in the series investigated by Grossman and Saward, the pools were larger (average of 50 donors). The survey of Scheinberg *et al.* was limited to a one-year period when the incidence of jaundice may possibly have been higher than was usual.

As mentioned before, we do not believe that it is possible to draw definite conclusions by comparing the results of different surveys. It seems sufficient to conclude from the various surveys including our own, that hepatitis may be transmitted by transfusion. Hepatitis is apparently a rare complication following transfusion of whole blood but its incidence is significantly increased by use of pooled plasma. Further, it seems justified to conclude that the larger the pool of plasma, the greater the chance for transmission of hepatitis.

If it is accepted that hepatitis is a definite complication, the question of prophylactic measures becomes of paramount importance. Obviously the first measure is to limit selection of donors to those who are less likely to transmit the disease. We have arbitrarily decided to reject all donors who have had jaundice within two years. Consideration should also be given to methods of sterilization of blood and/or plasma. Ultra-violet radiation, x-radiation and nitrogen mustard hold promise.[12] Of these, carefully controlled treatment by ultra-violet light, has been shown to sterilize plasma effectively without significant damage[2] but the necessary apparatus is sufficiently complicated and expensive to make it impractical for small hospitals. Such apparatus approved by the National Institute of Health is manufactured by three different organizations.*

The question whether to use pooled plasma or unpooled plasma is one which deserves careful consideration, if the material is not sterilized. Pooling almost certainly increases the hazard and there is fairly general agreement that large pools should be avoided. However, the safety of unpooled plasma as compared to plasma from small pools is more apparent than real. If a patient needs plasma, he usually needs at least two units of 250 cc. each, and often more. Consequently, the only advantage to the use of unpooled plasma may be nullified by what may be considered *in vivo* pooling as a result of multiple transfusions. Furthermore, the definite practical advantages of pooling must be considered. The fact that pooled plasma may be given without the necessity of blood grouping and Rh

* Sharp and Dohme Company, Glenolden, Pennsylvania; Michael Reese Research Foundation, 2912 Ellis Avenue, Chicago, Illinois; Mr. John J. Dill, 523 Terrace Court, Kalamazoo, Michigan (in cooperation with Dr. J. T. Tripp, Michigan Department of Health, Lansing, Michigan).

typing, is an advantage not only to the laboratory but to the attending physicians and patients as well. The use of unpooled plasma necessitates a certain amount of delay prior to administration and, in cases of severe shock, this may be an important factor in the survival of the patient. It is our opinion that small pools are the best compromise. Whenever patients receive multiple transfusions, the plasma should be taken from the smallest number of pools possible.

The use of plasma fractions, such as albumin, has been advocated in place of plasma for those patients in whom only the albumin fraction is needed. Even the cost of ultra-violet sterilization of plasma is far less than that of albumin fractionation and there seems to be little justification for this procedure except in research. If hepatitis ever becomes such a frequent complication of plasma transfusion that only sterilized plasma is used, it would seem much more feasible to develop regional centers for plasma production and sterilization rather than attempt fractionation. The immune bodies contained in the gamma globulin fraction of large pools of plasma seem to be of value in the prophylaxis of serum hepatitis when administered in sufficient quantity fairly early in the incubation period.[11] However, it is not practical to use this relatively scarce material for transfusion of every patient. There is no evidence that it is of value for treatment once the disease is established.

Finally, it may be well to point out that in almost all surveys in which an attempt has been made to estimate the incidence of hepatitis following plasma transfusion, the plasma that was used had been dried, frozen or at least refrigerated during storage. Freezing and drying are excellent methods for preserving viruses. It is possible that storage of plasma in the liquid state at room temperature might be harmful to the hepatitis virus and might even be a means of sterilization.

It is our plan to continue to use pooled plasma. We will continue to limit the size of the pools and not use more than 8 to 12 donors per pool. We hope to institute measures for virus sterilization as soon as possible and to make a second survey of the incidence of posttransfusion serum hepatitis later.

SUMMARY

1. A survey was made to determine the incidence of serum hepatitis following transfusions of blood and plasma over a four-year period beginning December 1943. Questionnaires were sent to 1,300 control patients and 1,523 patients who had been transfused with blood or plasma. Satisfactory responses were obtained from 1,000 controls and 936 transfused patients.

2. Among the 1,000 controls there were two patients (0.2 per cent) with "probable hepatitis".

3. Among the 936 transfused patients there were 20 (2.2 per cent) with "possible hepatitis" and 12 (1.3 per cent) with "probable hepatitis". Of 382 patients who received blood and no plasma, only one (0.3 per cent) had "probable hepatitis". These patients received a total of 3,723 transfusions of blood or plasma; an incidence of one case of "probable hepatitis" per 300 units of transfused blood or plasma. Of the 936 transfused patients, 528 received a total of 2,351 units of

plasma, with or without whole blood. Ten of these 528 patients had "probable hepatitis", an incidence of one case of hepatitis for every 235 plasma transfusions.

4. The significance of our findings and those of other surveys is discussed.

REFERENCES

1. The Outbreak of Jaundice in the Army: J. A. M. A. 120, 20: 51–53, 1942.
2. Blanchard, M. D., Stokes, J., Jr., Hampill, B., Wade, J. R., and Spizizen, J.: Methods of protection against homologous serum hepatitis. II. The inactivation of hepatitis virus SH with ultraviolet rays. J. A. M. A., 138: 341–343, 1948.
3. Brightman, I. J., and Korns, R. F.: Homologous serum jaundice in recipients of pooled plasma. J. A. M. A., 135: 268–272, 1947.
4. Grossman, C. M., and Saward, E. W.: Homologous serum jaundice following administration of commercial pooled plasma; report of eight cases including one fatality. New England J. Med., 234: 181–183, 1946.
5. Neefe, J. R.: Recent advances in the knowledge of "virus hepatitis". M. Clin. North American, 30: 1047–1443, 1946.
6. Neefe, J. R., Gellis, S. S., and Stokes, J., Jr.: Homologous serum hepatitis and infectious (epidemic) hepatitis; studies in volunteers bearing on immunological and other characteristics of the etiological agents. Am. J. Med., 1: 3–22, 1946.
7. Oliphant, J. W.: Infectious hepatitis; experimental study of immunity. Pub. Health Rep., 59: 614–616, 1944.
8. Sawyer, W. A., Meyer, K. F., Eaton, M. D., Bauer, J. H., Putnam, P., and Schwentker, F. F.: Jaundice in Army personnel in the western region of the United States and its relation to vaccination against yellow fever. Am. J. Hyg., 39: 337–430, 1943.
9. Scheinberg, I. H., Kinney, T. D., and Janeway, C. A.: Homologous serum jaundice; a problem in the operation of blood banks. J. A. M. A., 134: 841–848, 1947.
10. Spurling, N., Shone, J., and Vaughan, J.: The incidence, incubation period and symptomatology of homologous serum jaundice. Brit. M. J., 2: 409–412, 1946.
11. Stokes, J., Jr., Blanchard, M., Neefe, J. R., Gellis, S. S., and Wade, G. R.: Methods of protection against homologous serum hepatitis. I. Studies on the protective value of gamma globulin in homologous serum hepatitis SH virus. J. A. M. A., 138: 336–341, 1948.
12. Strong, L. E.: Summary of methods proposed for removing virus activity from protein solutions. Presented at conference on the preservation of the formed elements and of the proteins of the blood. Harvard Medical School, Jan., 1949.

THE COAGULATION MECHANISM

With Specific Reference to the Interpretation of Prothrombin Time and a Consideration of the Prothrombin Consumption Time *

ARMAND J. QUICK, M.D.

From the Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wisconsin

Until recently the hemorrhagic diseases remained in the realm of medical obscurities chiefly because adequate and specific laboratory tests were lacking. It might be well to comment on the procedures which were available to study these diseases fifteen years ago. The best known was the coagulation time which had nearly as many modifications as there were laboratories. Even today the coagulation time done on the same blood may vary from ten minutes to two hours, depending on the procedure used. It remains essentially a qualitative test which may often be normal, even though a serious defect in the coagulation mechanism actually exists. The coagulation time of recalcified oxalated plasma, sometimes referred to as Howell's prothrombin time, did not gain much recognition and contributed little to the understanding of the bleeding diseases. Three procedures, the bleeding time, clot retraction time and the tourniquet test were routinely carried out in most clinical laboratories, but they were most useful in confirming rather than in making the diagnosis of thrombocytopenic purpura. The only clotting agents for which quantitative methods were available were calcium, fibrinogen and platelets; and since no bleeding tendency is ever caused by calcium deficiency and only rarely by a diminution of fibrinogen, only the platelet count found clinical application.[1]

It is easy to understand the utter confusion that existed in the field of the bleeding diseases prior to 1934. Reputable investigators were questioning the very existence of prothrombin, and thromboplastin was a term even less well understood. In that year, in association with Bancroft and Stanley-Brown, I developed a quantitative method for determining prothrombin. We found that this factor was diminished in many patients with obstructive jaundice. Two years later I showed by means of this method that the prothrombin was diminished in vitamin K deficiency and in sweet clover disease. These were the first accurate quantitative studies and they clearly indicated that prothrombin had to be drastically reduced before a hemorrhagic tendency developed.[1]

At the same time that we developed this method for prothrombin, Smith, Warner, and Brinkhous reported in 1934 another quantitative procedure which required two steps and, therefore, has been designated as the two-stage procedure. They published the details of the method in 1936. In their hands it yielded important results, but owing to its intrinsic technical difficulties, it has not been

* Read before the Section on Clinical Pathology of the Medical Society of New Jersey, April 25, 1949.

Received for publication, July 13, 1949.

widely used in clinical laboratories. However, I wish to present the laboratory approach to the hemorrhagic diseases on the basis of our simple one-stage test and its recent offspring, the prothrombin consumption test.

To understand fully the physiologic process of blood coagulation and the part it plays in the hemostatic mechanism of the organism, it is advantageous to present it in the form of a simple diagram (Fig. 1) which incorporates the major known reactions.[2, 5]

Platelets on disintegrating liberate an enzyme which activates the inactive precursor thromboplastinogen to thromboplastin. The latter immediately interacts with the prothrombin complex to form thrombin, an enzyme, which promptly converts fibrinogen to fibrin and, even more important, acts upon the platelets to increase their speed of disintegration. This initiates an autocatalytic or chain-reaction which is all important in hemostasis as will be discussed subse-

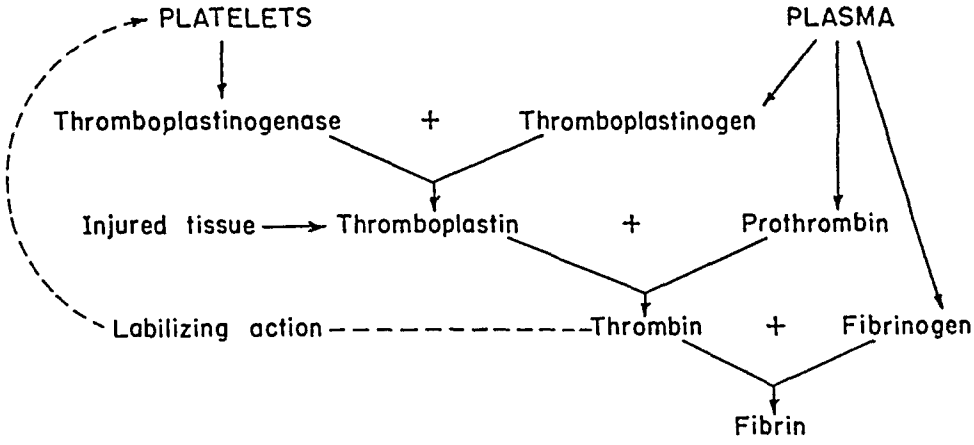


Fig. 1. Diagram of mechanism of blood coagulation

quently. Heretofore, the emphasis has been placed to a large extent on the formation of fibrin, which can roughly be measured by the coagulation time, but it now becomes clear that the key-substance in hemostasis is thrombin. By measuring the amount formed in coagulation, the true hemostatic efficacy of blood can be determined.

The direct estimation of thrombin formation by the two-stage method is technically difficult. The one-stage method is based on the simple reaction:

$$\text{Thromboplastin} + \text{Prothrombin Complex} = \text{Thrombin}$$

By using an excess of thromboplastin, the amount of thrombin formed is dependent directly on the concentration of prothrombin. A normal prothrombin time, therefore, is dependable evidence that the concentration of prothrombin is normal and that, therefore, the production of thrombin will also be normal, provided the supply of thromboplastin in the blood is adequate. The prothrombin time test, therefore, measures the prothrombin activity but furnishes no information concerning the thromboplastin concentration in the plasma. To de-

termine the value of this agent, the prothrombin consumption test was developed. The method consists in allowing blood to coagulate in a test tube and, after fixed periods of time, determining how much prothrombin remains in the serum. If the prothrombin consumption is normal, it indicates that both the platelets and the concentration of thromboplastinogen are normal.

By means of these two tests the clotting power of blood can be quantitatively appraised and, on this basis, the common hemorrhagic diseases can be logically classified into two principal classes, (1) the hypoprothrombinemias and (2) the thromboplastin deficiencies.

THE HYPOPROTHROMBINEMIAS

A prolonged prothrombin time indicates that the prothrombin activity of the blood is diminished. Recent studies have shown that bound calcium and three separate components constitute the prothrombin complex. While it may be of academic interest to argue which of these factors is the true classical prothrombin, it is of relatively minor clinical importance. It is far more practical to know that when the prothrombin time is prolonged to 20 seconds (which corresponds to a prothrombin activity of 25 per cent), irrespective of which component is responsible, a hemorrhagic tendency develops, as has been shown both experimentally and clinically. It should also be mentioned that only Component A requires vitamin K for its synthesis and, therefore, only the acquired types, caused by a deficiency of this factor, respond to vitamin-K therapy.

The hypoprothrombinemias can be classified as follows:

I. Congenital.[3] Three types have been described

II. Acquired
 - A. Lack of vitamin K
 1. Dietary deficiency
 - a. Hemorrhagic disease of the newborn
 2. Sterilization of intestinal tract by sulfonamides or antibiotic drugs
 3. Faulty absorption
 - a. Obstructive jaundice
 - b. Biliary fistula
 - c. Sprue
 - B. Liver damage
 1. Atrophic cirrhosis
 2. Hepatitis
 - C. Toxins
 1. Dicumarol
 2. Salicylates
 3. Antibiotics

The congenital types of hypoprothrombinemia are exceedingly interesting, but clinically of relatively little importance since they are rare. They may be confused with hemophilia, but the differential diagnosis is simple since the prothrombin time in hemophilia is usually normal and only occasionally slightly prolonged. None of the congenital types respond to vitamin K, but the prothrombin

can easily be elevated above the hemorrhagic level by plasma transfusion. One type is definitely hereditary and two persons both of whom have congenitally lowered prothrombin levels, should not inter-marry.[4]

Since vitamin K is abundantly present in all green vegetables and is synthesized by the intestinal bacteria, hypoprothrombinemia from dietary deficiency is rare except during the first few days of life. The newborn infant usually has little stored reserve of vitamin K and, therefore, may develop a marked hypoprothrombinemia for a few days after birth. The series which Grossman and I studied included several infants with prothrombin values as low as 5 per cent. These infants do not bleed spontaneously, but a slight injury may precipitate a fatal hemorrhage. A few tablets of vitamin K given to the mother during the last week of pregnancy is all that is needed to prevent this sinister threat to the newborn.

Since it is probable that much of the required vitamin K is derived from intestinal bacteria, care should be taken when giving drugs, such as the sulfonamides or antibiotics for sterilizing the intestinal tract, to be on the alert for the possible development of hypoprothrombinemia. It is always advisable, when treating a person for enteritis with antibacterial drugs, to administer vitamin K, preferably by the parenteral route.

Faulty absorption of vitamin K because of lack of bile salts in the intestinal tract is responsible for the hemorrhagic condition that may develop in obstructive jaundice or in biliary fistula. Since the advent of vitamin K, cholemic bleeding seldom occurs. It is interesting to note that in sprue, folic acid, which specifically increases intestinal absorption, may bring about a complete restoration of the prothrombin level of the blood. In atrophic cirrhosis the prothrombin level falls, but rarely enough to cause hemorrhage; vitamin K has no effect. Usually the prothrombin concentration remains constant for long periods, especially when the condition is quiescent. In acute hepatitis, on the contrary, the prothrombin may drop precipitously to very low levels and again rise rapidly as the liver recovers. In acute yellow atrophy the bleeding tendency is undoubtedly caused by the severe hypoprothrombinemia, although the reduction in fibrinogen may be a contributing factor. As in cirrhosis, vitamin K is completely ineffective. To what extent unrecognized liver damage, as in tuberculosis or metastatic malignancy of the liver, may be responsible for unexplainable hypoprothrombinemia, can only be conjectured.

The most common type of hypoprothrombinemia today is the one induced by Dicumarol, the exact action of which is still unknown. It is generally held that it interferes with the synthesis of prothrombin although some have postulated that it acts as an antivitamin, *i.e.*, the organism utilizes it in place of vitamin K and, therefore, produces an inert product instead of active prothrombin.

Since the problem of Dicumarol is far too involved to permit extensive discussion in this brief review, only a few important clinical facts can be mentioned. The therapeutic action of the drug depends solely on its ability to lower the prothrombin level of the blood, thereby reducing the potential quantity of thrombin that can be formed. The ideal level of prothrombin to be attained with Dicu-

marol is 20 per cent of normal. To determine the prothrombin accurately, I recommend that my method be carried out as I have described. The many modifications that have been suggested have only made results confusing.

Vitamin K is relatively ineffective in counteracting the hypoprothrombinemia of Dicumarol; therefore, when employed to elevate the prothrombin level, large doses must be employed and given preferably by vein. In general the recommended dose is 60 mg. of Hykinone (Abbott). Excessive lowering of prothrombin by Dicumarol can usually be prevented, if the initial dose does not exceed 300 mg., and the subsequent dosages controlled by daily prothrombin-time determinations until the maintenance dose is accurately determined. It is probable that Dicumarol is stored in the body and, therefore, large doses may cause a hypoprothrombinemia for as long as two weeks or until the stored material is exhausted.

There are, no doubt, a considerable number of other drugs, such as the salicylates, which may depress prothrombin activity. Their action is erratic, for in the majority of patients, the prothrombin level is only slightly lowered even after continuous administration of large doses of sodium salicylate. In a small group of patients, however, even moderate doses may cause a hypoprothrombinemia sufficiently severe to cause bleeding. Whether the action of the salicylates is the same as that of Dicumarol remains a moot question. Recently, it has been reported that penicillin may cause a marked hypoprothrombinemia. It is not known whether it acts directly on the synthesis of prothrombin or whether it depresses the growth of the intestinal bacteria, thereby curtailing the available supply of vitamin K.

HYPOTHROMBOPLASTINEMIA

Thromboplastin is as essential for the formation of thrombin as is prothrombin. By means of the prothrombin consumption test, the amount of available thromboplastin can be determined.[2] This test depends on ascertaining how much prothrombin remains after coagulation has been completed. Normally, enough thromboplastin is available to convert 85 per cent or more of the prothrombin. If the prothrombin consumption is incomplete, the cause is almost always a lack of thromboplastin which in turn is brought about either by a diminution of platelets, or by a deficiency of thromboplastinogen as in hemophilia. There is one exception observed thus far, namely, the appearance of an inhibitor of the platelet enzyme causing a hemophilia-like disease. The hypothromboplastinemias can be classified tentatively as follows:

Hypothromboplastinemias

A. Platelet factor dysfunction
 1. Thrombocytopenia
 2. Qualitative platelet defect (evidence still uncertain)
 3. Platelet enzyme inhibitor (hemophilia-like disease)

B. Hypothromboplastinogenemia
 1. Hereditary hemophilia
 2. Acquired (evidence still lacking)

In the practical application of the prothrombin consumption test, it was found that the original technic of allowing blood to stand for one hour after coagulation lacked sensitivity and did not always yield consistant results. Because of our recent finding that fibrin acts as a powerful adsorbant of nascent thrombin and prevents the initiation of the chain-reaction, the following technic was evolved.

The drawn blood is distributed in 2 ml. portions to 4 pyrex test tubes (100 x 13 mm.). These are placed in a water bath at 37 C. At the end of 25 minutes, the first tube is centrifuged in an International Clinical centrifuge for exactly one minute and an additional one-half minute is required to stop the centrifuge. The prothrombin of the serum is determined immediately and again in 15, 30 and 45 minutes. At the end of 40 minutes, the second tube

TABLE 1

PROTHROMBIN CONSUMPTION TIME OF NORMAL, HEMOPHILIC AND THROMBOCYTOPENIC BLOOD

TYPE OF SUBJECT	TUBE	MINUTES AFTER FORMATION OF SOLID CLOT			
		15	30	45	60
		Prothrombin Consumption Time in Seconds			
Normal	1	8*	26	33	32
	2		12*	28	34
	3			14*	28
	4				15*
Hemophilic	1	9*	9	9	9
	2		8½*	9	9
	3			9*	9
	4				9*
Thrombocytopenic	1	8*	9	9½	10½
	2		9*	10	12
	3			8*	10
	4				8*

* Immediately after centrifugation.

is centrifuged for one minute and the prothrombin of the serum determined as soon as the tube is removed from the centrifuge and again in 15 and 30 minutes. Likewise, tubes 3 and 4 are centrifuged at 50 and 70 minutes, respectively.

The results obtained from normal, hemophilic and thrombocytopenic bloods are summarized in Table 1.

It will be observed that the prothrombin time of serum immediately after centrifugation is very short, usually less than 10 seconds, but in normal serum it quickly increases, thus showing a rapid diminution of prothrombin. In hemophilic serum no such increase in the prothrombin time of serum occurs since thromboplastinogen is lacking. Likewise in thrombocytopenia, very little prothrombin is consumed and, therefore, the prothrombin time of serum does not increase rapidly on standing. It should be recognized that the prothrombin time of serum is uncorrected; the thrombin present at the time that the test is performed has an additive effect, thus causing the prothrombin time to be shorter

than the time required by the prothrombin alone. There is a direct relationship between the speed of prothrombin consumption and the number of platelets.

It is obvious from the data presented that the prothrombin consumption test offers the clinical laboratory an important new diagnostic procedure. It is valuable in the diagnosis of hemophilia. Often the clotting time, owing to technical errors, is normal especially in young children from whom it is difficult to obtain blood without a little admixture of tissue juice. The test promises to become particularly useful in the evaluation of the therapeutic effect of various agents in hemophilia. In the past too much credence has unjustifiably been given to changes in the coagulation time as a measure of therapeutic response. In thrombocytopenic purpura, the prothrombin consumption test can complement and perhaps at times even replace the platelet count. Since the test is still new and, therefore, has not been applied widely, its potentialities remain unpredictable but may perhaps be far-reaching.

SUMMARY

On the basis that thrombin formation is the key factor in hemostasis, two tests, the prothrombin time and the prothrombin consumption time, have been developed to supply the means for diagnosing and classifying the common hemorrhagic diseases resulting from coagulation defects. The prothrombin time measures dysfunctions of the prothrombin complex, while the prothrombin consumption test determines the amount of thromboplastin available in the blood. The principal diseases in which a lack of thromboplastin occurs are thrombocytopenia and hemophilia.

THE PROTHROMBIN CONSUMPTION TEST

Principle

Blood is allowed to coagulate under standardized conditions. The prothrombin remaining in the serum is determined by a technic similar to the original prothrombin method, except that fibrinogen is supplied in the form of deprothrombinized plasma.

Reagents

The same reagents as in the original prothrombin time method are employed. (1) calcium chloride 0.02 M, (2) thromboplastin (acetone-dehydrated rabbit brain): 0.2 Gm. is incubated with 5 ml. normal saline at 50 C. for 20 minutes, (3) deprothrombinized plasma: 9 volumes of freshly drawn human blood are mixed with one volume of 0.1 M sodium oxalate. The plasma is obtained by centrifugation. The prothrombin is removed by means of tricalcium phosphate. This is accomplished as follows: A measured volume of an 0.008 M suspension of tricalcium phosphate (1 ml. for every ml. of oxalated plasma to be treated) is transferred to a test tube. By centrifuging, the gelatinous calcium phosphate is packed and the surplus water poured off. The required volume of fresh oxalated human plasma is added, mixed with the adsorbent and repeatedly stirred with a small glass rod for 5 minutes at room temperature. The calcium phosphate is removed by centrifugation, and the clear adsorbed plasma poured into a clean test tube. Recently, it has been found that rabbit plasma because of its high content of labile factor is a more satisfactory reagent than human plasma. To deprothrombinize, 2.5 ml. of 0.005 M tricalcium phosphate is required for 1 ml. of rabbit plasma.

The Prothrombin Consumption Time Test

Determination of prothrombin time of serum. The deprothrombinized plasma (0.1 ml.) is mixed with 0.1 ml. thromboplastin and 0.1 ml. of 0.02 M calcium chloride. Into this mixture 0.1 ml. of the serum is blown by means of a serologic pipet and the time required for a clot to form accurately determined.

Technique of prothrombin consumption test. Blood obtained by venipuncture is distributed in 2 ml. portions to 4 test tubes (100 × 13 mm.). These are placed in a water bath at 37 C. The time required to form a solid clot is noted and 15 minutes later the tube is put in an International Clinical centrifuge and spun at full speed for one minute. After an additional one-half minute required to stop the centrifuge, the prothrombin time is immediately determined and again for three consecutive 15-minute periods. Thirty minutes after coagulation, the second tube is centrifuged and the prothrombin time of the serum determined immediately and for two additional 15-minute periods. At the end of 45 minutes, following coagulation the third tube is taken out of the water bath, centrifuged and the prothrombin determined immediately and in 15 minutes. At 60 minutes after centrifugation, the prothrombin time is determined in the fourth tube. The third tube may be omitted.

Directions for preparing tricalcium phosphate. 158 Gm. of trisodium phosphate are dissolved in 1000 ml. of distilled water. Separately, 66.6 Gm. of anhydrous calcium chloride are dissolved in 1000 ml. of distilled water. The calcium chloride solution is poured into the trisodium phosphate with thorough stirring. The pH is adjusted to 7. The precipitated tricalcium phosphate is washed repeatedly by decantation until the sodium chloride is removed. The volume is brought to 1 l. thus making a 0.2 M suspension. From this stock a 0.005 M solution is prepared by mixing 2.5 ml. with 97.5 ml. of distilled water.

REFERENCES

1. Quick, A. J.: The coagulation defect in sweet clover disease and in the hemorrhagic chick disease of dietary origin; a consideration of the source of prothrombin. Am. J. Physiol., **118:** 260-271, 1937.
2. Quick, A. J.: The Hemorrhagic Diseases and the Physiology of Hemostasis. Springfield, Illinois: Charles C Thomas, 340 pp., 1942.
3. Quick, A. J.: On the constitution of prothrombin. Am. J. Physiol., **140:** 212-220, 1943.
4. Quick, A. J.: On the quantitative estimation of prothrombin. Am. J. Clin. Path., **15:** 560-566, 1945.
5. Quick, A. J.: Studies on the enigma of the hemostatic dysfunction of hemophilia. Am. J. M. Sc., **214:** 272-280, 1947.
6. Quick, A. J.: Components of the prothrombin complex. Am. J. Physiol., **151:** 63-70, 1947.
7. Quick, A. J.: Congenital hypoprothrombinemia and pseudo-hypoprothrombinemia. Lancet, **2:** 379-382, 1947.
8. Quick, A. J., and Grossman, A. M.: The nature of the hemorrhagic disease of the newborn; delayed restoration of the prothrombin level. Am. J. M. Sc., **199:** 1-9, 1940.
9. Quick, A. J., Shanberge, J. N., and Stefanini, M.: The role of platelets in the coagulation of the blood. Am. J. M. Sc., **217:** 198-205, 1949.
10. Quick, A. J., Stanley-Brown, M., and Bancroft, F. W.: A study of the coagulation defect in hemophilia and in jaundice. Am. J. M. Sc., **190:** 501-511, 1935.
11. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Titration of prothrombin in certain plasmas. Arch. Path., **18:** 587, 1934.
12. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: A quantitative study on blood clotting; prothrombin fluctuations under experimental conditions. Am. J. Physiol., **114:** 667-675, 1936.

EFFECT OF ULTRAFILTRATION AND CARBON DIOXIDE ON ANTITHROMBIN ACTIVITY OF FRESH AND STORED HUMAN PLASMA*

MARIO STEFANINI, M.D.†

From the Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wisconsin

In a previous communication,[11] it was shown that the antithrombin titer of human plasma rises during storage. This effect is directly related to the degree of decalcification, since it is pronounced when plasma is decalcified with well purified Amberlite IR-100, 0.01 M sodium oxalate, or 0.02 M sodium citrate, and less evident when calcium is only partially removed by treatment with incompletely purified Amberlite or 0.01 M sodium citrate.[12] Subsequently, an effort was made to find a technic capable of limiting or altogether preventing the rise of antithrombin activity of plasma during storage.

Chak and Giri[2] have presented evidence that passage of carbon dioxide and ultrafiltration restore the decreased prothrombin activity of human plasma that has been held for periods up to one week. Their results suggested the study of the effect of similar procedures on the antithrombin activity of fresh and stored human plasma. Ultrafiltration, adsorption of plasma with calcium phosphate [$Ca_3(PO_4)_2$] gel and treatment with various gases (CO_2, O_2 and N_2) were investigated.

MATERIALS AND METHODS

1. Oxalated or citrated blood was obtained by mixing 9 volumes of venous blood with 1 volume of 0.1 M sodium oxalate or 0.1 or 0.2 M sodium citrate; and "Amberlite blood", by passing 10 ml. of venous blood through a column of 3 Gm. of the resin Amberlite IR-100 in the sodium cycle, as outlined in a previous communication.[10] Plasmas were obtained by centrifuging the blood at 2000 r.p.m. for 5 minutes.

2. Plasmas were transferred to unstoppered Pyrex tubes (130 x 10 mm.) and refrigerated at constant temperature of 4 C.

3. The plasma was passed through a sterilized, dry Seitz ultrafilter, under negative pressure.

4. CO_2, O_2 and N_2 from an ordinary gas machine were passed through the plasma for 3 minutes, according to the technic described by Quick.[3]

5. For adsorption of plasma, 0.008 M of $Ca_3(PO_4)_2$ gel was used. The technics for the preparation of the gel and for the adsorption of plasma have been previously described.[7]

6. The prothrombin time was determined with the one-stage method of Quick.[5]

7. The concentration of the labile factor was determined with the one-stage procedure of Quick and Stefanini.[6]

* Received for publication, July 26, 1949.

† Present address, Joseph H. Pratt Diagnostic Hospital, Boston 11, Massachusetts.

8. The antithrombin activity of plasma was determined by the "incubation method", recommended by the author,[11] as follows: Full-strength thrombin was used, obtained from fresh oxalated human plasma, according to the method of Quick.[4] One-tenth ml. of the preparation (which may be kept in the frozen state for at least one month without any appreciable loss of potency) clotted 0.2 ml. of fresh normal oxalated human plasma (source of fibrinogen) in three seconds. Equal volumes of full-strength thrombin and plasma under investigation were incubated in a water bath at 37 C. The clot formed was removed by wrapping it about a glass rod coated with collodion. At regular one-minute intervals for five minutes, 0.1 ml. of the mixture was added to 0.2 ml. of fresh oxalated human plasma and the clotting time recorded. The values obtained for normal oxalated,

TABLE 1

ANTITHROMBIN ACTIVITY OF VARIOUSLY DECALCIFIED AND NATIVE FRESH HUMAN PLASMA*
(Average results of several experiments)

	TIME OF INCUBATION OF THE THROMBIN-PLASMA MIXTURE, MINUTES				
	1	2	3	4	5
	Clotting Time, Seconds				
Plasma, native	8	9	12	16.5	22.5
Oxalated (sodium oxalate 0.01 M)	14	21	62	457	∞
Citrated (sodium citrate 0.02 M)	15	27.5	78	571	∞
Citrated (sodium citrate 0.01 M)	12.5	14	16	21.5	28.5
Treated with fully active Amberlite	13	17	52	384	∞

* Equal volumes of full strength human thrombin and plasma under investigation were incubated in water bath at 37 C. The clot formed was immediately removed by wrapping it about a glass rod coated with collodion. At one-minute intervals for five minutes, 0.1 ml. of the mixture was added to 0.2 ml. of normal fresh oxalated human plasma and the clotting time recorded.

citrated and Amberlite plasma are shown in Table 1. This technic has a greater sensitivity than the direct method under the experimental conditions described. Errors caused by possible changes in reactivity of fibrinogen during storage are not likely to occur since fibrinogen obtained from human plasma stored for 30 days is still acted upon normally by thrombin.[11]

RESULTS

(a) *Effect of different procedures on the antithrombin titer of fresh, variously decalcified human plasma.* Passage of N_2 or adsorption by 0.008 M $Ca_3(PO_4)_2$ gel did not cause any change in the antithrombin titer of fresh plasma. The latter result confirms earlier findings of Astrup and Darling.[1] Instead, the antithrombin activity was evidently reduced by ultrafiltration and passage of CO_2 and O_2. The effect was less marked in the more completely decalcified plasmas, which originally presented higher antithrombin titer. The results obtained are summarized in Table 2. On the contrary, the prothrombin time of plasma was only minimally

affected by ultrafiltration or passage of CO_2, but variably delayed by treatment with O_2. This last effect was due to a partial destruction of the labile factor, as was indicated by the direct determination of this component. The absolute con-

TABLE 2

EFFECT OF DIFFERENT PROCEDURES ON THE ANTITHROMBIN TITER OF FRESH, VARIOUSLY DECALCIFIED HUMAN PLASMA*

	TIME OF INCUBATION OF THE THROMBIN-PLASMA MIXTURE, MINUTES		
	1	2	3
	Clotting Time, Seconds		
0.01 M oxalated plasma	14	21	62
Ultrafiltered	8.5	11	19
Treated with CO_2	11	15	24
Treated with O_2	12.5	19	42
Treated with N_2	14	20.5	64
Adsorbed with $Ca_3(PO_4)_2$ gel	15.5	22	68
0.02 M citrated plasma	15	27.5	76
Ultrafiltered	10.5	17	26
Treated with CO_2	12	22	39
Treated with O_2	14	27	51
Treated with N_2	16	29	78
Adsorbed with $Ca_3(PO_4)_2$ gel	18	35	97
Amberlite plasma	13.5	17	52.5
Ultrafiltered	8	10.5	18
Treated with CO_2	10	14.5	22
Treated with O_2	12	17	38
Treated with N_2	14	19	57
Adsorbed with $Ca_3(PO_4)_2$ gel	15	24	65
0.01 M citrated plasma	12.5	14	16.5
Ultrafiltered	5.5	7	9.5
Treated with CO_2	6.5	8.5	11
Treated with O_2	10	14	18
Treated with N_2	12	14.5	21
Adsorbed with $Ca_3(PO_4)_2$ gel	13	16	24

* Equal volumes of full strength human thrombin and plasma under investigation were incubated in water bath at 37 C. The clot formed was immediately removed by wrapping it about a glass rod coated with collodion. At one-minute intervals for three minutes, 0.1 ml. of the mixture was added to 0.2 ml. of normal fresh oxalated human plasma and the clotting time recorded.

centration of prothrombin, as determined by the method recently introduced by Quick and Stefanini,[8] was not reduced by any of the procedures mentioned.

Experiments were also conducted to determine whether treatment with CO_2 or ultrafiltration of fresh plasma could effectively prevent the rise of antithrombin titer occurring during storage. The results are presented in Table 3. Apparently O_2, N_2 and adsorption with $Ca_3(PO_4)_2$ gel did not affect the increase of antithrombin activity during storage. This was, on the other hand, limited by treating fresh plasma with ultrafiltration or CO_2. Ultrafiltration showed a stronger

limiting effect on the rise of antithrombin activity of stored plasma than CO_2. If, however, plasmas were stored in an atmosphere of CO_2, the rise in antithrombin titer was comparable to that observed in the ultrafiltered sample. With the same procedure, the loss of labile factor during storage was strikingly reduced. A sample of oxalated human plasma stored in an atmosphere of CO_2 was found to contain 20 per cent of the original concentration in labile factor after six months.

TABLE 3

EFFECT OF ULTRAFILTRATION AND TREATMENT WITH CO_2 OF FRESH HUMAN PLASMA ON THE RISE OF ANTITHROMBIN ACTIVITY DURING STORAGE*

Plasmas stored for 5 days

	TIME OF INCUBATION OF THE PLASMA-THROMBIN MIXTURE, MINUTES		
	1	2	3
	Clotting Time, Seconds		
0.01 M oxalated plasma	19	39	92
Ultrafiltered	12.5	25	56
Treated with CO_2, in stoppered container	14	28	64
Treated with CO_2, in opened container	16	34	80
0.02 M citrated plasma	20	42	103
Ultrafiltered	14	29	72
Treated with CO_2, in stoppered container	15.5	32	78
Treated with CO_2, in opened container	16.5	36	89
Amberlite plasma	18	35	78
Ultrafiltered	11	22	50
Treated with CO_2, in stoppered container	12.5	24	58
Treated with CO_2, in opened container	13.5	27	68
0.01 M citrated plasma	13.5	15.5	17.5
Ultrafiltered	6.5	8	11
Treated with CO_2, in stoppered container	7	10.5	13
Treated with CO_2, in opened container	8	10.5	13

* Equal volumes of full strength human thrombin and plasma under investigation were incubated in water bath at 37 C. The clot formed was immediately removed by wrapping it about a glass rod coated with collodion. At one-minute intervals for three minutes, 0.1 ml. of the mixture was added to 0.2 ml. of normal fresh oxalated plasma and the clotting time recorded.

(b) *The effect of different procedures on the antithrombin titer of stored, variously-decalcified plasma.* Plasmas decalcified by addition of 1/10 volume of 0.1 M sodium oxalate, 0.1 or 0.2 M sodium citrate or by passage through Amberlite were stored for ten days at 4 C. After centrifugation for five minutes at 2000 r.p.m. to eliminate the fine floccules formed under these conditions, the plasmas were treated with ultrafiltration, adsorption with $Ca_3(PO_4)_2$ gel or various gases. Some of the results are summarized in Table 4. Ultrafiltration and, to a lesser extent, passage of CO_2 evidently reduced the antithrombin titer of stored plasma. Passage of O_2 had only a minimal effect while passage of N_2 and adsorption by $Ca_3(PO_4)_2$ gel were practically ineffective. When the plasmas so treated were

stored again, the antithrombin titer also rose again, but at a slower rate than in control plasmas. The different treatments did not affect the concentration of labile factor or the prothrombin time of the stored plasmas.

TABLE 4

EFFECT OF DIFFERENT PROCEDURES ON THE ANTITHROMBIN TITER OF VARIOUSLY DECALCIFIED HUMAN PLASMAS, STORED FOR TEN DAYS AT 4 C.*

	TIME OF INCUBATION OF THE THROMBIN-PLASMA MIXTURE, MINUTES		
	1	2	3
	Clotting Time, Seconds		
0.01 M oxalated plasma	26	52	125
Ultrafiltered	12.5	26	59
Treated with CO_2	16.5	39	88
0.02 M citrated plasma	29	57	154
Ultrafiltered	14	32	75
Treated with CO_2	17.5	38	94
Amberlite plasma	24	46	112
Ultrafiltered	11	22	48
Treated with CO_2	14	29	62
0.01 M citrated plasma	14.5	17	19
Ultrafiltered	7.5	9.5	12.5
Treated with CO_2	8	10.5	14

* Equal volumes of full strength human thrombin and plasma under investigation were incubated in water bath at 37 C. The clot formed was immediately removed by wrapping it about a glass rod coated with collodion. At one-minute intervals for three minutes, 0.1 ml. of the mixture was added to 0.2 ml. of normal fresh oxalated human plasma and the clotting time recorded.

TABLE 5

EFFECT OF ULTRAFILTRATION AND CO_2 ON THE ANTITHROMBIN ACTIVITY OF BANK BLOOD STORED FOR TWENTY DAYS*

	TIME OF INCUBATION OF THE THROMBIN-PLASMA MIXTURE, MINUTES				
	1	2	3	4	5
	Clotting Time, Seconds				
Stored plasma	13	15.5	19	25	34
Ultrafiltered	6	7.5	10.5	15	18
Treated with CO_2	8	11	14.5	18	24

* Equal volumes of full strength human thrombin and plasma under investigation were incubated in water bath at 37 C. The clot formed was immediately removed by wrapping it about a glass rod coated with collodion. At one-minute intervals for five minutes, 0.1 ml. of the mixture was added to 0.2 ml. of normal fresh oxalated human plasma and the clotting time recorded.

(c) *The effect of ultrafiltration and CO_2 on the properties of bank blood.* Ten samples of blood stored for transfusion were obtained from the Junior League Blood Center of Milwaukee through the courtesy of Dr. Tibor Greenwalt, Medical Director. The blood had been collected in sterile vacuum containers (Baxter

Laboratories, Morton Grove, Ill.), using as anticoagulant and preservative a citrate-dextrose solution* in the proportion of 120 ml. of the solution for 380 ml. of blood, and stored at 4 C. for 20 days. The blood was then centrifuged twice at 2000 r.p.m. for ten minutes. The plasma obtained appeared in a good state of conservation. Both fibrinogen level and concentration of prothrombin were found to be normal; the prothrombin time, however, was found to be delayed from a normal value of 12 to between 25 and 34 seconds, owing to the decreased concentration of the labile factor (from 25 to 15 per cent of the original normal level). Under these conditions of collection and storage, the rise of antithrombin titer was limited. Moreover, as shown by the results presented in Table 5, it was drastically reduced by ultrafiltration and, to a lesser extent, by passage of CO_2, while the concentration of labile factor and of prothrombin remained unmodified.

DISCUSSION

The previously reported finding that the antithrombin activity of plasma increases during storage in direct relationship with the grade of decalcification has been confirmed. The elevation of antithrombin titer becomes evident only after the fifth day of storage, so that the phenomenon has no bearing on the determination of the prothrombin time as obtained with the one-stage[5] or the two-stage technic.[14, 15]

The finding that ultrafiltration and passage of CO_2 will decrease the antithrombin titer of stored plasma and partly prevent its rise during storage might prove to be a useful tool in the study of the significance and properties of natural antithrombin. Unfortunately, an explanation of the mechanism by which this effect is achieved cannot be suggested at present. Particularly difficult to explain is the observation that the rise of antithrombin activity during storage is much less pronounced in plasmas ultrafiltered or treated with CO_2 than in untreated control samples. The observation would suggest that the two procedures partially destroy not only actual antithrombin but also a precursor from which more antithrombin is formed during storage.

The finding that an increase of antithrombin activity occurs in blood and plasma collected in the usual citrate-dextrose solution and stored for transfusion purposes has some practical significance. Of course, the effect is moderate, as would be expected since the concentration of sodium citrate in bank blood is approximately 0.01 M. Moreover, the elevated antithrombin titer can be reduced to practically a normal level by ultrafiltration or treatment with CO_2 of the plasma.

The question arises whether the moderately increase in antithrombin activity of blood after prolonged storage has any practical significance. It appears that if the blood or plasma are used within the first seven to ten days of storage, the increase of antithrombin activity is obviously not great enough to limit the therapeutic effect of the transfusion. If, however, the blood has been stored for longer than two weeks, the increased antithrombin titer might be of some im-

* 100 ml. of the solution contain: dextrose, 2.45 Gm.; sodium citrate, 1.37 Gm.; citric acid, 0.5 Gm.; distilled water, to volume.

portance. Transfusion of such blood may cause a temporary and limited rise in the plasmatic antithrombin of the recipient, as we have observed in preliminary experiments to be reported later. Further work is in progress for better evaluation of this effect which could assume clinical significance when blood is given to patients receiving heparin or a circulating anticoagulant. In these conditions, it might be advisable to ultrafilter the blood or plasma or treat them with CO_2, preliminary to transfusion.

SUMMARY

1. The antithrombin activity of plasma rises during storage in direct relationship with its degree of decalcification. The phenomenon is evident only after the plasma has been stored from five to ten days.

2. Ultrafiltration and treatment with carbon dioxide reduce the antithrombin titer of both fresh and stored plasma. Treatment with oxygen has a similar, but limited, action; treatment with nitrogen or adsorption of plasma with calcium phosphate gel does not produce any significant effect. If plasma is first treated with ultrafiltration or CO_2 and then stored, the increase of its antithrombin activity is less pronounced than in an untreated sample.

3. Blood collected in citrate-dextrose solution for transfusion purposes shows a limited rise of antithrombin activity during storage, which can be reduced to almost normal values by ultrafiltration or passage of CO_2. When this blood is transfused after a period of storage longer than two weeks, the plasmatic antithrombin-titer of the recipient is slightly and temporarily raised. This effect may present clinical significance only in patients receiving heparin or having a circulating anticoagulant. In these clinical conditions preliminary ultrafiltration or treatment with CO_2 of the blood or plasma to be transfused might be advisable.

4. Ultrafiltration and CO_2 do not affect the absolute concentration of prothrombin or of the labile factor in stored plasma. Treatment with O_2, by inactivating variable amount of the labile factor, will cause prolongation of the prothrombin time.

REFERENCES

1. Astrup, T., and Darling, S.: Measurements and properties of antithrombin. Acta physiol. Scandinav., **4**: 293-308, 1942.
2. Chak, I. M., and Giri, K. V.: Effect of ultra-filtration and CO_2 on the prothrombin activity of plasma. Nature, **159**: 878-879, 1947.
3. Quick, A. J.: Effect of air currents on plasma prothrombin. Proc. Soc. Exper. Biol. and Med., **50**: 317-318, 1942.
4. Quick, A. J.: The Hemorrhagic Diseases and the Physiology of Hemostasis. Springfield, Ill.: Charles C Thomas, 1942, 340 pp.
5. Quick, A. J.: On the quantitative estimation of prothrombin. Am. J. Clin. Path., **15**: 560-566, 1945.
6. Quick, A. J., and Stefanini, M.: The concentration of the labile factor of prothrombin in human, dog and rabbit blood; its significance in the determination of prothrombin activity. J. Lab. and Clin. Med., **33**: 819-826, 1948.
7. Quick, A. J., and Stefanini, M.: The chemical state of calcium reacting in the coagulation of blood. J. Gen. Physiol., **32**: 191-202, 1948.
8. Quick, A. J., and Stefanini, M.: The concentration of component A in blood; its assay and relation to the labile factor. J. Lab. and Clin. Med., **34**: 973-982, 1949.
9. Quick, A. J., and Stefanini, M.: The state of component A (prothrombin) in human blood. Evidence that it exists partly free and partly in an inactive or precursor form. J. Lab. and Clin. Med., in press.

10. STEFANINI, M.: Purification of the resin Amberlite IR-100 for blood coagulation studies. Proc. Soc. Exper. Biol. and Med., **67**: 22–25, 1948.
11. STEFANINI, M.: Antithrombin activity of stored plasma. Am. J. Clin. Path., **18**: 537–541, 1948.
12. STEFANINI, M.: Studies on the role of calcium in the coagulation of blood. Acta med. Scandinav., in press.
13. STEFANINI, M. AND QUICK, A. J.: Effect of various adsorbants on the components of the prothrombin complex. Fed. Proc., **8**: 255, 1949.
14. WARE, A. G., AND SEEGERS, W. H.: Two-stage procedure for the quantitative determination of prothrombin concentration. Am. J. Clin. Path., **19**: 471–482, 1949.
15. WARNER, E. D., BRINKHOUS, K. M., AND SMITH, H. P.: A quantitative study on blood clotting; prothrombin fluctuations under experimental conditions. Am. J. Physiol., **114**: 667–675, 1936.

A SURVEY OF THE ACCURACY OF Rh ANTIBODY TITRATIONS IN SEVERAL HOSPITAL LABORATORIES*

ROGER W. MARSTERS, PH.D.

From the Maternity Rh Laboratory of the University Hospitals of Cleveland, Cleveland, Ohio

Davidsohn and Stern[2] have stated that tests for Rh antibodies occupy a unique position because of their complete specificity and high sensitivity. The achievement of a high level of reliability, however, is more difficult than is generally appreciated, inasmuch as tests for Rh antibodies are delicate and quite subject to gross error. We have had the opportunity to follow the blood anti-Rh concentration of many immunized women through several pregnancies, and of hyperimmunized experimental subjects for a number of months. Some of the titer changes, which we formerly considered as significant, we know now were caused solely by variation of method. Some modifications have been introduced into the original technic in our attempts to increase the accuracy of the method. We have spent considerable time rechecking specimens giving equivocal results or an end point significantly different from that previously found. Recently we have adopted some of the National Institute of Health technics for blood-group antibody titrations. It is important to standardize the entire technic and to use standard Rh-positive red cells of known agglutinability. These standard cells should be titered occasionally against known serums, and are most reliable when the blood is freshly drawn from the donor. It is essential that one technician have the responsibility for running all the titers and that she be adequately supervised and checked to insure reliable results.

Our technic for this determination is based upon that first suggested by Abelson and elaborated upon and published by Diamond and Denton.[4] This is the now-familiar titration-technic using doubling dilutions of the patient's "unknown" serum with saline, adding a small quantity of a 2 per cent saline suspension of Rh-positive red cells to an equal quantity of each dilution, incubating and reading. Bovine albumin or its equivalent is then substituted and the final reading made.

Because of our experiences with Rh-antibody titrations, we wondered how other laboratories were faring with these determinations. It seemed of interest to attempt a survey of the Rh-antibody technic of several laboratories running this test routinely. Upon inquiry, each laboratory was willing to submit its reports of titrations on duplicate bloods and to cooperate in a joint study. All members of the group fully understood the details of the survey and ran the submitted specimens exactly as any routine blood for anti-Rh titer. Since then, the data presented here have been submitted to the individual laboratories, and the factors responsible for the observed discrepancies have been discussed and presumably corrected. Publication of these data seemed desirable because of the

* Received for publication, July 11, 1949.

probability that some other laboratories making this determination might be led to check their technics for possible inaccuracies. MacCready and McGee[5] have pointed out the occurrence of unsuspected errors in routine Rh-typing in hospital laboratories. Their data indicate inaccuracy in a significant percentage of bloods tested for the Rh factor (about 7 per cent of a series of almost 1500 Rh tests). As might be expected, certain laboratories were committing a considerable number of errors, while other laboratories had almost perfect accuracy.

Van Saun[6] has emphasized the apparent lack of technical competency in many laboratories and has pointed out the desirability of a central public health laboratory taking over the responsibility for routine Rh-blood testing. Routine Rh-antibody titrations have usually been left to the research laboratory, but some hospital laboratories are now making this determination. The tests are usually run in the blood bank. The present survey was undertaken in order to evaluate the success of such an arrangement.

METHODS

Over a six-week period, a total of 15 specimens of whole blood or serum was distributed to each of the cooperating laboratories. Laboratories A, B, C, and D are hospital laboratories that do not specialize in determination of Rh-antibody titration but perform this test on request. Laboratory E is our own laboratory while Laboratory F is directed by a well-known original investigator in the Rh field. All specimens for distribution were prepared at the same time and dispatched on the same day except for some specimens that were sent late to Laboratories D and F, but this apparently did not affect the results. The blood specimens were obtained by means of a large syringe either by withdrawal, directly from the patient, or from a sterile flask of serum stored at 4 C. They were aliquotted into sterile Wassermann tubes for distribution. The specimens were numbered consecutively and labelled with fictitious names.

One of the specimens distributed was not of human origin but was taken from a goat being used for an unrelated study. This animal's serum showed non-group specific agglutinins; more than a dozen different human bloods of various OAB groups and Rh (D) types were rapidly clumped by this serum both in saline and in albumin medium. This serum, containing heteroagglutinins, was distributed to determine if all titrations were being adequately controlled with Rh-negative cells. With this exception, the remaining specimens were obtained from known immunized Rh-negative women, each of whom had delivered one or more erythroblastotic offspring. Each of these patients was known to us and all had typical histories. None was pregnant at the time of taking the blood although four of them had their last erythroblastotic baby within a year, while a fifth carried a high titer all through the last pregnancy and delivered a normal Rh-negative child. Four other older women, with typical histories, have been artificially hyperimmunized.

RESULTS

One specimen of serum was distributed as Numbers 1, 4, 5, and 13 and the results are shown in Table 1. Specimen Numbers 4 and 5 were distributed to-

gether and presumably were run simultaneously. The end points reported from Laboratories A, C, and D for these two specimens show extreme variation and yet presumably both tests were performed by the same person using the same Rh-positive cells. Another serum specimen was distributed as Numbers 6, 11,

TABLE 1

RESULTS OF ANTI-RH TITRATIONS OF DUPLICATE BLOOD SPECIMENS TESTED BY DIFFERENT LABORATORIES

SPECIMENS TESTED	DILUTING MEDIUM	TITERS OBTAINED BY SIX DIFFERENT LABORATORIES					
		A	B	C	D	E	F
One serum distributed as No. 1, 4, 5, 13	Saline	0, 8, 8, 64	64, 64, 128, 256		8, 16, 64, 128	64, 128, 128, 256	64
	Human serum-bovine albumin	4, 32, 128, 256	128, 256, 256, 512	2, 4, 128, 256	16, 16, 16, 32	128, 128, 128, 256	128
One serum distributed as No. 6, 11, 14	Saline	0, 8, 64	64, 512, 512		16, 16, 32	1, 16,1 32	6, 32,
	Human serum-bovine albumin	32, 64, 128	512, 512, 2048	8, 64, 256	8, 8, 16	64, 128, 512	16, 512

TABLE 2

RESULTS OF ANTI-RH TITRATIONS OF BLOOD SPECIMENS TESTED BY DIFFERENT LABORATORIES

NO. OF SPECIMEN TESTED	TITERS* OBTAINED BY SIX DIFFERENT LABORATORIES					
	A	B	C	D	E	F
2	4/16	32/512	/2	0/8	1/256	16/128
3	4/64	16/32	/1	0/2	1/32	0/32
7	2/4		/4	2/0		
8	0/2	0/8	/0	0/0	1/1	0/32
9	16/32	128/256	/128	16/1	64/32	32/32
10	128/128	256/1024	/256	16/8	64/64	64/64
12	128/128	1024/2048	/256	128/256	32/256	512/512
15	64/128	256/2048	/128	32/16	32/128	8/64

* The figures preceding the diagonals are end points obtained with saline; the figures following the diagonals are end points obtained with serum-albumin.

and 14 and the results are also shown in Table 1. The titration end points are represented both for saline and albumin. There was poor grouping of the end points in either medium except for Laboratories B and E on the first resubmitted specimen. Only Laboratory B showed fair grouping on the second resubmitted specimen.

The results of Laboratory C were only given for one medium, and yet the variation was extreme. The results for Laboratory A on both resubmitted specimens ranged from saline end points of 1:64 to zero activity in saline. The close groupings of Laboratory D for the first serum and especially for the second serum are fortuitous. The goat serum, containing heteroagglutinins, was reported as containing Rh antibodies by Laboratories A, C, and D. No figure is shown for this specimen (Number 7) since the titration end point was of no significance.

The end points reported by the cooperating laboratories for the 7 remaining singly submitted serums are shown in Table 2. The several extremely low end points reported by Laboratories A, C, and D make it appear that they might have missed antibodies entirely in serums with only low to moderate anti-Rh titers. The only serum distributed with a low titer (Number 8) was actually reported as "negative for anti-Rh" by Laboratories C and D. There were no changes of personnel performing the tests or of technics as far as could be determined in any of the cooperating laboratories during the survey period.

DISCUSSION

Admitting that the different technics and positioning of end points employed might give different answers, it would seem that each laboratory should be consistent and capable of reproducing its former result. Although some variation in end point-reading is perhaps unavoidable, it would seem that anything short of absolute specificity would represent a technical error.

An examination of the reports submitted by the various laboratories indicates that Laboratory A showed a few end points in the low range, particularly in saline medium. Laboratory A's results on the resubmitted specimens did not show satisfactory grouping as can be seen in Table 1. Although this laboratory claimed to run a control with Rh-negative cells, it reported that the goat serum contained Rh antibodies. The reports of Laboratory A are, therefore, considered to be inaccurate.

Laboratory B immediately noted agglutination with the animal serum against Rh-negative cells and inquired if this was an Hr serum. This laboratory made the logical response to the animal serum and their other end point values are satisfactory, although somewhat in the high range on the last specimens. This tendency was found to be due to a change of donors during the survey. The trend to higher values was recognized by the technician and she was planning to find a more suitable donor, or if possible, to return to the original donor. Furthermore, the reproducibility of results was satisfactory and it is considered that Laboratory B was reliable.

Laboratory C did not routinely set its titrations up in saline medium. This is contrary to the usual practice and, in our experience, the changes in type of antibody are often as revealing as the absolute titration-end points. A patient with antibodies demonstrable only in albumin will often begin to show some activity in saline as she approaches term if the baby is Rh-positive. Laboratory C submitted extremely low reports for approximately half of all the specimens, showed

poor reproducibility on the duplicates, missed the antibodies entirely in Specimen No. 8, and reported the heteroagglutinins of the goat serum "Rh antibodies". The reports of Laboratory C were believed to be of doubtful value.

Laboratory D also failed to detect the antibodies in Specimen Number 8 and reported the goat serum positive for Rh antibodies. The reports of this laboratory showed much variation and questionable reproducibility. It submitted a copy of its data sheet for each titration and an examination of these data shows atypical findings. A solid "four plus" clump will, in our experience, usually drop to a "three plus" or perhaps a "two plus" in the next higher dilution tube, but never to zero as was reported by that laboratory. It was difficult to decide exactly where its end point occurred because occasionally, after the agglutination had apparently been "diluted out", the next higher dilution was reported as "two plus". Apparently the tubes were being agitated too vigorously and the negatives were not checked under the microscope. This laboratory also considered the serum dilution after the blood suspension had been added. This was contrary to usual practice. For comparison we have taken this into consideration and the reports of Laboratory D are based on serum dilutions before the addition of cell suspension. The reports of Laboratory D were undoubtedly subject to serious technical error.

Laboratory E submitted end point figures which compared well with those of Laboratories B and F. The goat serum showed tight "four plus" clumping both in saline and albumin with negative as well as positive cells and was, therefore, recognized as nonspecific. With one exception, the reproducibility of its results was satisfactory, and it detected the low-titered anti-Rh in Specimen Number 8.

Laboratory F did not receive all the specimens studied. The end points reported were apparently comparable to those submitted by Laboratories B and E. With one possible exception, its end points were satisfactory.

The terminology of the cooperating laboratories was noted. Laboratory C employed the term "agglutination" but set its titers up in AB serum only and did not check for saline agglutinins. Laboratories A, B, and D employed the terms "agglutinating" and "conglutinating" for the Rh antibodies demonstrable with saline and albumin. We, as well as Laboratory F, employ the general term "agglutination" and qualify it as suggested by Davidsohn[1] to indicate the particular medium employed, *i.e.*, "saline agglutinin" or "serum-albumin agglutinin". The use of the term "serum-albumin agglutinin" seems preferable to any other because this term clearly designates the type of test done. We do not use the term "blocking antibody" because this carries the connotation of "blocking action" which we are not measuring. We do not use the blocking test routinely nor does any other laboratory included in this survey. The descriptive terms "early immune" and "hyper-immune" originally proposed by Diamond[3] for the saline-active and albumin-active antibodies are excellent and fitting, but the terms suggested by Davidsohn are perhaps more suitable from a technical point of view.

In reporting end points, Laboratories A and D were employing the least

amount of grossly-visible clumping as the end point, with the tendency to disregard any fine clumping, while Laboratories B, C, and F were reading the end point microscopically. We have considered the end point as the very last dilution tube which shows grossly-visible agglutination. This is the "one plus" end point of the National Institute of Health procedure and is more reproducible than the microscopic end point which may be found in the tube one to three dilutions higher. In practice, we always check the "one plus" tube as well as the tube with the next-higher dilution under the microscope to be sure of the positioning of the one-plus end point. Occasionally a titration will drop from a "two plus" to a "plus-minus" in which case, we consider the "plus-minus" to be the end point. Two of the laboratories which reported some very low end points occasionally mentioned that "rouleaux formation" was present in these specimens in tubes of higher dilution. Apparently they were confusing rouleaux formation with agglutination.

The medium employed by the cooperating laboratories for the demonstration of the serum-albumin agglutinin was also recorded. Laboratories A and F employed 20 per cent Bovine Albumin,* while Laboratories C and D used AB serum.† Laboratory B used the plasma from its standard cell donor, while we employed 30 per cent bovine albumin mixed with an equal volume of AB serum. This mixture has been used in our laboratory for some time and we believe that it gives more clear-cut results than either medium alone. The mixture seems to demonstrate Rh antibodies much better than does AB serum alone, and yet diminishes the occasional hemolysis and "sticky" trace of clumping caused by some lots of bovine albumin. Diamond and Denton[4] have mentioned some of the objections to the use of plasma as a suspension medium. It does not seem that the different mediums employed by the several laboratories could have been a significant factor in explaining the variety of serum-albumin end points reported.

It is interesting that the end points submitted by the laboratories which apparently were technically correct showed so much variation. Both Laboratories B and E detected the animal serum, both found the low titer on Specimen Number 8, and their groupings on the resubmitted specimens were comparatively excellent. However, an occasional end point reported by each of Laboratories B, E, and F must necessarily be considered out of range of acceptable results. Thus, it would appear that even under ideal conditions, an occasional titration will be unsatisfactory. It is interesting that the laboratories with unsatisfactory end points on the duplicate specimens were using random cells for their titrations. In our experience, not all donors are suitable and we often examine several individuals before finding one whose cells will give an expected end point with a known antiserum and agglutination that is clear-cut. Furthermore, since most blood cells give decreasing titration end points as they age, the standard cells

* Bovine Albumin, Armour, 30 per cent (Special for Rh-testing), diluted with half volume of sterile saline before use.

† AB Serum (Hyland).

for titers should be less than 48 hours old. The practice of using random cells for Rh-antibody titrations should be discouraged.

When all technical details of the procedures have been carefully standardized, the variation between titration end points should not usually exceed one dilution tube. However, we no longer consider a trend between two titrations as significant until the end point continues to change to the next dilution tube in another titration. For example, if the end point changes from 1:16 to 1:32, we do not consider the titer as rising until a third determination gives an end point of 1:64. This emphasizes the need of determining the titer early in the pregnancy and following the titer at frequent intervals in patients who are known to be immunized.

SUMMARY

A survey was made of six hospital laboratories, including our own and a control laboratory, of the results of titration of Rh antibodies on identical specimens of blood serum. Only one of the four laboratories not specializing in this work, gave results that were considered to be reliable. The variation in results obtained by different laboratories technically without reproach was appreciable, and indicates the need for acceptance of, and rigid adherence to, one conventional technic for this determination.

Acknowledgment. The joint cooperation of the individual laboratories which made this survey possible is appreciated. I wish to thank Miss Helen Karlovec of this laboratory for technical assistance.

REFERENCES

1. Davidsohn, I.: Rh antibodies; correlation with clinical findings. Blood, Special Issue No. 2, January, 1948, pp. 139–154.
2. Davidsohn, I., and Stern, K.: Interpretation of Rh antibodies. Am. J. Clin. Path., **18**: 690–699, 1948.
3. Diamond, L. K.: The production and proper use of Rh typing reagents. Am. J. Pub. Health, **38**: 645–651, 1948.
4. Diamond, L. K., and Denton, R. L.: Rh agglutination in various media with particular reference to the value of albumin. J. Lab. and Clin. Med., **30**: 821–830, 1945.
5. MacCready, R. A., and McGee, R. T.: Blood grouping and Rh typing in a state public health laboratory. Am. J. Pub. Health, **38**: 637–644, 1948.
6. van Saun, A. I.: Is the Rh factor a public health laboratory function? Am. J. Pub. Health, **38**: 632–636, 1948.

INTERSTITIAL CELL TUMOR OF THE TESTIS

Report of Two Cases*

CHARLES R. REINERS, Jr., M.D., and ROBERT C. HORN, Jr., M.D.

From the Laboratory of Surgical Pathology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania

Chevassu,[3] in 1906, recorded the first description of a tumor arising from the Leydig or interstitial cells of the testis. In 1944, Nation, Edmondson and Hammack[11] collected 24 cases from the literature, adding two of their own. Subsequently, Scully and Parham[15] reported one case, bringing the total number of interstitial cell tumors recorded in the literature to 27.

In the extensive and thorough reviews of the literature of interstitial cell growths of the testis,[7, 11, 15, 22] there is no agreement as to the authenticity of all of the reported cases. We have reviewed the original reports of these cases and, with the possible exceptions of the following two instances, are inclined to agree with Nation and his associates[11] in their appraisal. It appears likely that the case of Sharnoff and Lisa[16] represents hyperplasia of interstitial cells in a patient with an advanced carcinoma of the prostate, and that the lesion described by Snellman[17] is a genuine neoplasm of Leydig cells.

During a two and one-half year period only six neoplasms of the testis were encountered in the Laboratory of Surgical Pathology of the Hospital of the University of Pennsylvania; and of these, two were interstitial cell tumors. However, among 33 testicular tumors previously reviewed in this laboratory,[13] no interstitial cell tumors were recognized. This incidence (5 per cent) is higher than that reported by Friedman and Moore[4] (1 per cent). The discrepancy is perhaps accounted for, at least in part, by the fact that Friedman and Moore were dealing with younger men of military age while many of the reported interstitial cell tumors have occurred in older persons. Nevertheless, it seems possible that some interstitial cell tumors may not have been recognized as such, primarily because of their infrequent occurrence. There has been a difference of opinion with regard to the diagnosis in our Case 1, although the tumor meets all the diagnostic criteria. Furthermore, the practice of giving radiation therapy preoperatively to testicular tumors suspected of being malignant has greatly complicated the problem of histologic diagnosis in many instances,[13] and conceivably might have so altered some interstitial cell tumors thusly treated, as to render them unidentifiable. The normal Leydig cell, however, is generally considered to be resistant to the effects of radiation.[21]

In view of the infrequent recognition of this type of tumor and, because of the controversy over the possible interstitial cell origin of some tumors, as well as over the distinction between hyperplasia and neoplasia of interstitial cells,[22] it seems worthwhile to report two interstitial cell tumors that we have studied,

* Received for publication July 25, 1949.

emphasizing especially those pathologic features which should permit the recognition of this group of neoplasms.

REPORT OF CASES

Case 1

Clinical Data. A thirty-four year old white male, was admitted to the University Hospital on January 14, 1947, with the complaint of a hard, pea-sized, painless nodule in the upper portion of the left testicle noted six weeks previously. His local physician advised observation. In the subsequent six weeks the lump more than doubled in size, and at the time of admission measured 3 cm. in diameter. Six days before admission he had noted a dull pain along the dorsum of his left ankle, leg and thigh, which subsequently involved the medial aspect as well.

The *past history* included mumps at the age of ten. There had been no accompanying orchitis. Four years before this admission, the patient had struck the left testicle while leaping onto a bicycle. He had been nauseated for several hours thereafter, but there were no other known sequelae. Sexual development had been normal. He was married and the father of two children, seven years and three and one-half years old. After the nature of his tumor was known, questioning elicited the fact that beginning six months before admission, the patient had had an intensely overactive sexual drive. This had continued until one month before admission, when his libido decreased markedly.

Physical examination was negative with the exception of the left testicle. There was no abnormal breast development and the body hair was normally distributed. The penis and right testis were normal. In the region of the upper pole of the left testis there was a firm, non-tender mass about 3 cm. in diameter. The lower pole of the testis and the vas deferens were normal to palpation. Rectal examination was negative. No inguinal nodes were palpable and there were no abdominal masses.

Laboratory data. Complete blood count and urinalysis were within normal limits. Roentgenographic examination of the chest and of the urinary tract (by intravenous urography) showed no significant changes. Positive findings on roentgenographic study included evidence of an old, healed, depressed skull fracture and a small area of calcification in the right testis. Only a soft-tissue density was noted in the left testicle. A qualitative Friedman test was negative. The urinary gonadotropin level was 79 mouse units in 24 hours, and two months later was 168 mouse units per 24 hours (normal, 4–24 mouse units per 24 hours). The 17 ketosteroids were determined only postoperatively (fifth day). The level was 10.5 milligrams in 24 hours. This last figure is within normal limits.

Treatment. On January 16, 1947, left orchiectomy was performed by Dr. P. Boland Hughes. The spermatic cord and vessels were ligated at the level of the external inguinal ring and, following this, the cord, epididymis and testicle were excised. Postoperatively the patient did well and was discharged on the seventh postoperative day with the wound well healed. Despite a lack of precedent for treatment of interstitial cell tumors, it was decided to employ postoperative roentgen therapy in the manner advocated by Pendergrass[13] for testicular tumors in general. A total of 3650 r units in air was given, equally divided over four 15 x 20 cm. portals, anteriorly over the mediastinum, and anteriorly and posteriorly over the lumbar spine. The factors were 200 Kv., 15 MA, 50 cm. focal distance and filters of 1 mm. of aluminum and 0.5 mm. of copper. After a dose of only 200 r was delivered over the right lower quadrant of the abdomen, treatment was discontinued on February 14, 1947 because of a fall of the white blood count to 1750.

Course. Eighteen months postoperatively, a chest roentgenogram, an intravenous urogram and the value of estrogen excreted in the urine were all within normal limits. On May 30, 1949, twenty-eight months after operation, the patient was well and without clinical evidence of active disease.

Pathologic description. The specimen consisted of a testis together with the



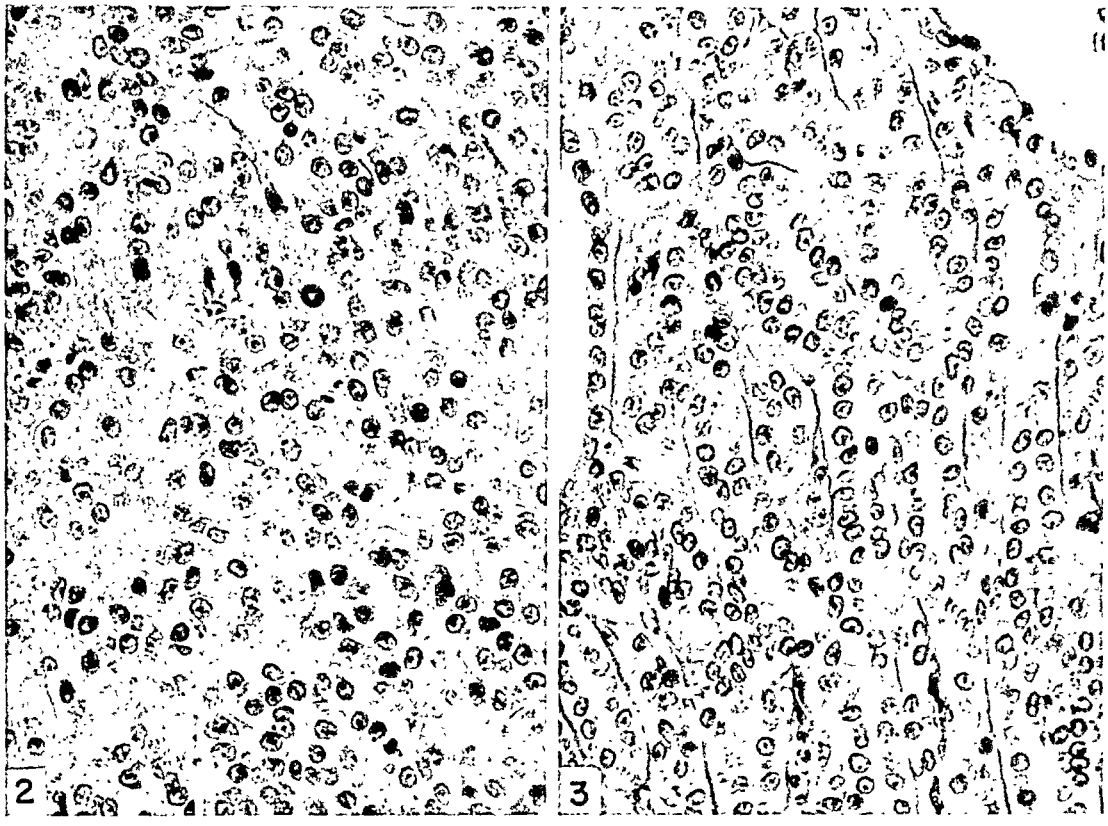


FIG. 1. Case 1. Operative specimen. The testicle and tumor have been bisected. The round, well-circumscribed tumor is in the upper pole of the testicle. The lobulated appearance of the tumor is well demonstrated.

FIG. 2. Case 1. The general pattern of the tumor simulates that of many tumors of endocrine glands. H. and E. × 350.

FIG. 3. Case 1. This area shows an unusual glandular pattern which was observed in only a small part of the tumor. Above, the cells lie in cords along thin-walled capillaries, simulating the architecture of liver or adrenal cortex. H. and E. × 350.

epididymis and spermatic cord. Only the testis showed any gross abnormality. It measured 4.5 cm. in diameter and was soft and tan. In the upper pole was a soft, solid, well circumscribed tumor 2.5 cm. in diameter, the cut surface of which was yellow-gray and homogeneous. The tumor was entirely within the body of the testis, the covering layers being intact (Fig. 1).

Microscopically, the tumor was highly cellular, the stroma being scanty and highly vascular. There was an incomplete capsule, beneath which remnants of compressed, atrophic seminiferous tubules were occasionally mingled with the tumor. In some areas, testicular tissue and tumor were seen side by side, without any evidence of an intervening capsule. Throughout most of the tumor the cells were closely packed in solid sheets without any particular arrangement (Fig. 2), but a distinct pattern was evident in some areas. The most striking pattern was one of orderly rows of cells, arranged with their long axes perpendicular to the rows, bordering fibrous trabeculae. At times, indefinite anastomotic cords of cells could be made out, simulating the structure of hepatic cell cords (Fig. 3). In other areas, the cells were divided by fibrous bands into small alveolus-like clusters. The cells were moderately large and polygonal, usually with well marked cell boundaries. The nuclei were round to oval, and varied in size and shape. Some were quite small, while others almost completely filled the cell. Multinucleated cells were occasionally seen. The cytoplasm was faintly acidophilic, sometimes granular, and often vacuolated. Abundant lipoid material was demonstrated within many of the cells with the Scharlach R stain. Mitotic figures were noted infrequently, approximately one in every 6–8 high power fields. The cells also bore a certain resemblance to liver cells. Brown pigment, giving the iron reaction, was present within many macrophages in the stroma.

Sections of testicle at a distance from the lesion showed numerous seminiferous tubules with active spermatogenesis. Interstitial cells did not appear increased. Sections of the spermatic cord showed no evidence of tumor.

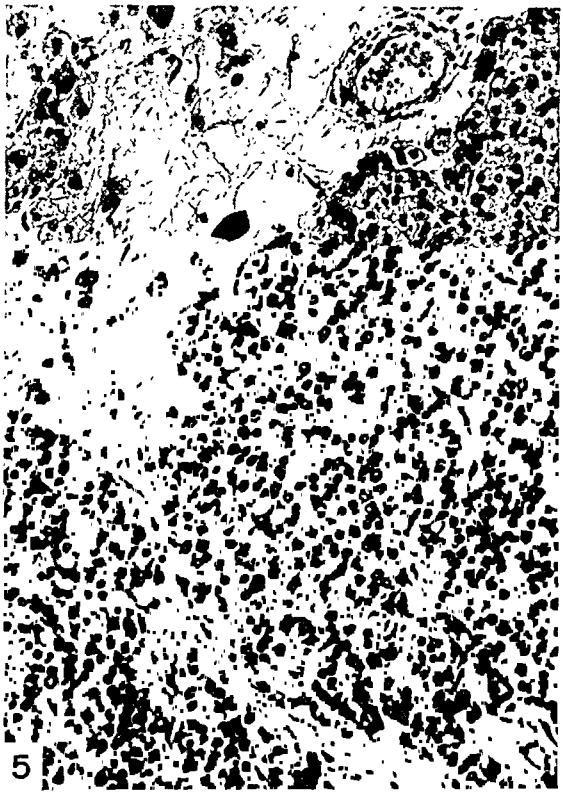
Diagnosis. Interstitial cell tumor of the testis.

Case 2

Clinical data. A fifty-four year old negro, was referred to the Urology Clinic of this hospital November 15, 1948 from the Gastro-Intestinal Clinic where he had been under treatment for duodenal ulcer. He was somewhat vague about dates, but stated that his chief complaint was pain in the right testicle which he had first noticed three years before. The pain was of a dull aching type and did not radiate. The attacks came on at 2 or 3 day intervals without apparent cause, lasted several hours and then spontaneously disappeared. In the past six weeks the pain had been more noticeable, and the testicle had been sore between attacks. At no time had this pain been severe, and he never sought medical aid prior to this clinic admission. The testicle had never been hot, red, or swollen.

Past history. The diagnosis of duodenal ulcer had first been made five years before. Symptoms had been moderately well controlled by diet. The patient gave a history of having had gonorrhea on two occasions, the second time four years before admission, when he had been treated with sulfadiazine. He had been treated from 1942 to 1945 for syphilis in the Syphilology Clinic of the University Hospital. The past history was otherwise negative. The patient was married and had one child sixteen years of age. He had noticed no change of libido or sex power, and had intercourse on the average of once a week up to the time of admission. He was employed as a truck driver.





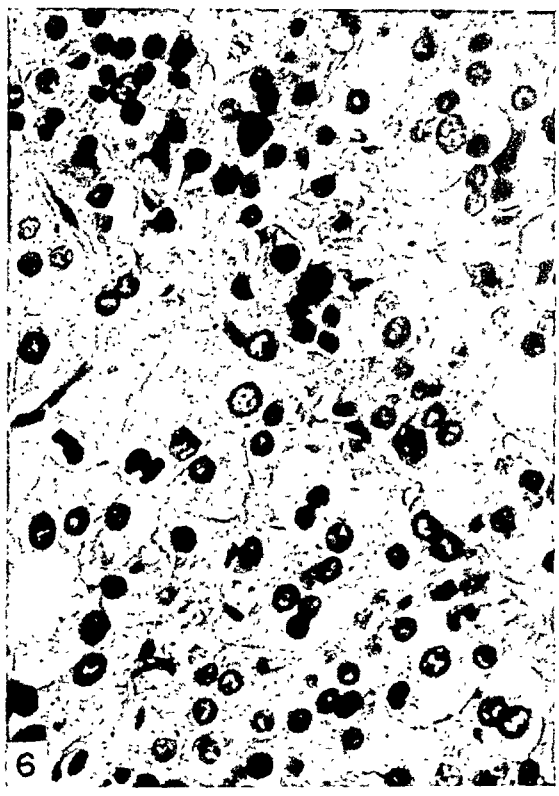


FIG. 4. Case 2. The tumor occupies over half the bulk of the testicle. Here, again, the nodular pattern is well shown.

FIG. 5. Case 2. The cells are arranged in no specific pattern. H. and E. × 150.

FIG. 6. Case 2. The tumor cells are conspicuously vacuolated. These vacuoles were demonstrated to contain lipoid material. This photomicrograph also illustrates the nuclear variability. H. and E. × 400.

Physical examination was negative except for the genitalia. The penis was of normal size and appearance. The right testicle was slightly larger than the left and was hard, nodular, and slightly tender. There was no abnormal distribution of body hair or hypertrophy of the breasts. The inguinal lymph nodes were not enlarged. There was slight non-tender enlargement of the prostate.

Laboratory data. Blood count, urinalysis, and blood urea nitrogen, and blood sugar values were normal; Kolmer test, four-plus; Kahn test, positive; Kline test, positive (four units); cerebrospinal fluid, Wassermann 0000, protein 50 mg., colloidal mastic 1111000000. Roentgenographic examinations of the chest and urinary tract (intravenous urogram) were negative. There was a bullet in the right side of the abdomen the position of which varied in different roentgenograms. It was thought to be lodged in the gastrointestinal tract. Excretion of urinary gonadotropins was 29 mouse units in 24 hr., excretion of estrogen was less than 13 mouse units in 24 hours, and excretion of 17-ketosteroids 8.9 mg. in 24 hours. All of these figures may be considered to be within the limits of normal.

Treatment. On November 16, 1948, under local anesthesia, the right testicle, epididymis and spermatic cord were excised following preliminary ligation of the cord and vessels at the level of the external inguinal ring.

The postoperative course was uneventful and the patient was discharged on his eleventh postoperative day with the wound well healed. The patient is under observation for leukoplakia of the buccal cavity. Seven and one-half months after operation he revealed no evidence of recurrent or metastatic tumor.

Pathologic description. The specimen consisted of a testicle, epididymis and associated structures including the tunica vaginalis and spermatic cord. The testis measured 3 x 2.5 x 2 cm. and contained a tumor measuring 2 x 1 x 1.3 cm. The testis around the tumor was yellowish gray and pulpy and appeared normal. The tumor was nodular, soft and yellow-tan, distinct from the rest of the testis and enclosed by a thin, gray capsule. Invasion could not be seen grossly (Fig. 4). The tunica albuginea was intact. No abnormalities of epidymis or vas deferens were noted. A portion of the tumor, weighing 2.14 Gm., was analyzed by Dr. F. Curtis Dohan and found to contain 0.616 mg. of 17-ketosteroids.

Microscopically, the nodular character of the tumor was evident. Nests of tumor cells were separated by broad bands of relatively acellular connective tissue. The collagenous fibrils were frequently separated by small clear spaces. The tumor cells grew in solid sheets or cords (Fig. 5), occasionally showing a pattern reminiscent, as in the first case, of liver architecture. Resemblance to the structure of adrenal cortex was also suggested. The individual cells were large and well defined, generally with eccentric nuclei. Multinucleated cells were frequent. The cytoplasm was faintly acidophilic, finely granular, and in many of the cells, was vacuolated (Fig. 6). Occasionally, vacuolization was so extensive that the cells resembled foam cells. Occasional cells contained finely granular, brown pigment, but this was an inconstant finding. No other cytoplasmic inclusions were seen. The nuclei varied considerably in size and shape (Fig. 6) but, in general, were large and round. Most of the nuclei contained single prominent nucleoli. No mitotic figures were seen. Within the tumor nests the stroma was sparse. Thin-walled capillaries were seen lying beside, and in intimate relation with, the cords of tumor cells. There was no invasion of the tumor capsule or of the surrounding testis or vas deferens. In the sections of testicle outside the capsule of the tumor, the majority of seminiferous tubules appeared normal.

There was some patchy atrophy, but spermatogenesis was active. The interstitial cells did not appear to be increased.

Diagnosis. Interstitial cell tumor of testis.

DISCUSSION

The tumors reported fulfill the recognized criteria of interstitial cell tumors.[15] They do not resemble any of the more common testicular tumors, either grossly or microscopically. Both tumors were nodular, yellow or yellow-brown, and encapsulated, at least in part. The tumor in Case 2 was completely invested by a capsule and tumor tissue and seminiferous tubules were intermingled at some points; in Case 1 encapsulation was incomplete. Microscopically, both tumors were composed of polyhedral cells growing in sheets, cords, or clusters, separated by greater or lesser amounts of collagenous stroma. Not infrequently, cords of tumor cells were related to sinusoid-like vascular spaces in a manner suggestive of the architecture of liver or adrenal cortex. The cell cytoplasm was acidophilic and finely granular frequently containing vacuoles (shown by Scharlach R stain to represent lipoid droplets) and less often containing granules of brown pigment. The nuclei varied considerably in size and shape. Multinucleated cells were not rare. Large, punctate, occasionally multiple nucleoli were usually situated eccentrically in the nuclei. Mitotic figures were not observed in one tumor and they were infrequent in the other. The pathologic features of the typical interstitial cell tumor are sufficiently distinctive so that, in Case 2, we were able to make the correct diagnosis on the basis of gross examination and study of rapid frozen sections.

It does not seem necessary to consider the possibility that these lesions might represent hyperplasias rather than true tumors, because of their size, partial or complete encapsulation, discreteness and ready distinction from the testicle proper. The differentiation between hyperplasia and neoplasia does, however, present great difficulties in many instances.[22] Snellman[17] has suggested that such differentiation be made on the basis of size of the mass, proposing a diameter of 0.75 cm. as the arbitrary upper limit of an hyperplastic nodule. It would seem that encapsulation and microscopic pattern should represent equally important criteria.

In their review, Nation and his associates[11] report that clinical evidences of hormonal imbalance are infrequent except in children, where precocious sexual and bodily development is the rule. Gynecomastia occasionally occurs in the adult. Libido and sexual power may be increased, decreased or unchanged. These changes usually, but not invariably, regress after operative removal of the tumor. In our Case 1, the increase of libido was extreme, persisting over a five month period but decreasing in the last month prior to operation.

It is unfortunate that in so many of the reported cases hormone assays have been incomplete or have been entirely omitted, and that, therefore, definitive conclusions cannot be drawn. In the case of Hunt and Budd[2, 6] the Aschheim-Zondek reaction was strongly positive (1000 units of luteinizing hormone per liter of urine) and 20 units of estrin were demonstrated in 1.73 grams of tumor tissue.

Negative Aschheim-Zondek or Friedman tests have been reported in a number of cases.[1, 5, 19, 22, 23] Masson and Venning[10, 20] reported a case in which the excretion of gonadotropin and estrogen was slightly elevated (110 and 113 mouse units per day, respectively) and in which the urinary 17-ketosteroids were elevated to approximately fifty times normal (1040 mg. per day). Their determinations were not made at the time of primary admission but only when the patient returned to the hospital with extensive metastatic disease. Negative hormone determinations in our cases included urinary 17-ketosteroid excretion in both cases, Friedman test in Case 1 and estrogen excretion in Case 2. (The 17-ketosteroid determinations were done only postoperatively). Assay of the tumor tissue in Case 2 yielded but small amounts of 17-ketosteroids. The patient in Case 1 did show an increased urinary excretion of gonadotropins. Although the elevation was not great, it is of interest that it was this patient who had previously experienced a marked increase of libido.

Our information concerning the endocrine relationships of interstitial cell tumors is no doubt so incomplete because of the infrequency with which this tumor type is recognized and because, when the diagnosis is made, it is based on pathologic study. Masson's and Venning's opportunity[10, 20] to study a patient with metastatic disease years after the pathologic diagnosis had been established on the primary tumor is an exception. Complete data will become available only if hormone assays are routinely made a part of the study of all patients with possible tumors of the testis.

Four of the reported interstitial cell tumors have metastasized.[8, 9, 10, 11] It has been emphasized by Bonser and Hawksley[1] and by Warren and Olshausen[22] that the demonstration of metastases is the only certain criterion of malignancy. Many of the reported benign cases have shown pleomorphism, even bizarre cells, and many mitotic figures, but no metastases during the periods of observation ranging from six months to 10½ years. Local invasion of limited extent is also noted in many of the benign tumors. These features are seen so often that they might be considered characteristic of interstitial cell tumor. It might be remarked that these same features are not infrequently encountered in neoplasms of other endocrine glands which do not prove malignant. It is of great interest to note that in the case reported by Masson[9] there was a symptom-free period of nine years following extirpation of his tumor. At the end of this interval the patient returned to the hospital with extensive metastases and subsequently died. Masson and Sencert's[10] patient developed metastases four and one-half years after operation. The two other malignant cases developed metastases within a relatively shorter period of time.

Because of the lack of adequate histologic criteria of malignancy and in view of the fact that metastases may occur late, Waren and Olshausen[22] emphasize the importance of carefully following, for at least ten years, every patient whose testicle contains an increased number of interstitial cells. Needless to say, the period of follow-up in our two cases is too short to permit any assurance as to the benign nature of the tumors.

SUMMARY

Two cases of interstitial cell tumor of the testis are reported, bringing to 29 the total number of tumors of this type reported in the literature. The gross and microscopic pathologic features are emphasized. The deficiencies in our knowledge of interstitial cell tumors are perhaps greatest with respect to their endocrine relationships, deficiencies that can be corrected only by including careful hormone assays in the routine study of all patients having possible testicular neoplasms.

REFERENCES

1. Bonser, G. M., and Hawksley, L. M.: Two cases of interstitial cell tumor of the human testes. J. Path. and Bact., **55**: 295–299, 1943.
2. Budd, J. W.: Gynecomastia associated with interstitial cell tumor of the testis (abstract). Am. J. Path., **13**: 660–661, 1937.
3. Chevassu, M.: Tumeurs du testicule. G. Steinheil, Paris, 1906, pp. 35–39, p. 186; in Paris Theses, 1905–1906.
4. Friedman, N. B., and Moore, R. A.: Tumors of the testis. Mil. Surgeon, **99**: 573–593, 1946.
5. Huffman, L. F.: Interstitial cell tumor of the testicle: report of a case. J. Urol., **45**: 692–698, 1941.
6. Hunt, V. C., and Budd, J. W.: Gynecomastia associated with interstitial cell tumor of the testicle. J. Urol., **42**: 1242–1250, 1939.
7. Jemerin, E. E.: Hyperplasia and neoplasia of the interstitial cells of the testicle. Arch. Surg., **35**: 967–998, 1937.
8. de Josselin de Jong, R.: Ein Fall von Zwischenzellengeschwulst in einem ektopischen Hoden bei Pseudohermaphroditismus masculinus internus. Frank. Ztschr. f. Path., **34**: 420–431, 1926.
9. Masson, P.: Tumeur maligne des cellules de Leydig. Rev. canad. de biol., **1**: 570–571, 1942.
10. Masson, P., and Sencert, L.: Cancer des cellules interstitielles. Bull. Assoc. franc. p. l'étude du cancer, **12**: 555–572, 1923.
11. Nation, E. F., Edmondson, H. A., and Hammack, R. W.: Interstitial cell tumors of the testis. Report of three new cases. Arch. Surg., **48**: 415–422, 1944.
12. Neeve, R. H., and Marsh, F.: Interstitial-cell testicular tumors. J. Path. and Bact., **56**: 575–576, 1944.
13. Pendergrass, E. P., Chamberlin, G. W., Selman, J., and Horn, R. C.: The management of malignant tumors of the testis. Am. J. Roentgenol., **55**: 555–574, 1946.
14. Rowlands, R. P., and Nicholson, G. W.: Growth of the left testicle with precocious sexual and bodily development (macro-genito-somia). Guy's Hosp. Rep., **79**: 401–408, 1929.
15. Scully, R. E., and Parham, A. R.: Testicular tumors. II. Interstitial cell and miscellaneous neoplasms. Arch. Path., **46**: 229–242, 1948.
16. Sharnoff, J. G., and Lisa, J. R.: Malignant tumor of the interstitial cells of the testis with prostatic carcinoma. J. Urol., **50**: 471–473, 1943.
17. Snellman, B.: A case of tumor-like hyperplasia of the interstitial cells in the testis. Am. J. Cancer, **35**: 258–263, 1939.
18. Somerford, A. E.: A case of interstitial cell tumor of the testis in a boy of eleven years. Brit. J. Urol., **13**: 13–16, 1941.
19. Stewart, C. A., Bell, E. T., and Roehlke, A. B.: An interstitial-cell tumor of the testis with hypergenitalism in a child of five years. Am. J. Cancer, **26**: 144–150. 1936.
20. Venning, E. H.: Étude hormonale sur un cas de tumeur interstitielle du testicule. Rev. canad. de biol., **1**: 571–572, 1942.
21. Warren, S.: Effects of radiation on normal tissues. VIII. Effects on the gonads. Arch. Path., **35**: 121–127, 1943.
22. Warren, S., and Olshausen, K. W.: Interstitial cell growths of the testicle. Am. J. Path., **19**: 307–331, 1943.
23. Werner, A. A., Spector, H. I., Vitt, A. E., Ross, W. L., and Anderson, W. A. D.: Pubertas precox in a six year old boy produced by a tumor of the testis, probably of interstitial cell origin. J. Clin. Endocrinol., **2**: 527–530, 1942.

BOOK REVIEWS

Trends in Medical Education. The New York Academy of Medicine Institute on Medical Education, 1947. Edited by MAHLON ASHFORD, M.D. 320 pp., $3.00. New York: The Commonwealth Fund, 1949.

This volume includes papers presented and their resulting discussions at the New York Academy Institute on Medical Education in 1947. It is divided into the following sections:

1. Premedical Education and the Selection of Medical Students
2. Undergraduate Medical Education
3. The Medical Education of Interns and Residents
4. Graduate and Postgraduate Training for Specialization
5. The Responsibility of Medical Schools and Hospitals in the Education of the General Practitioner
6. The Relation of Group Practice and Community Medical Services to Medical Education.

Material on each of these six major sub-divisions was presented by experts in the particular field.

With respect to selection of students for the medical school, there seems to be no general agreement as to prerequisite courses of study. Some believe that emphasis should continue to be placed on scientific training, while others believe that premedical training is deplorably deficient in the cultural subjects. Thus, the statement, "The medical profession is a prisoner of its own success," implies that while medicine has made great strides in the scientific field, it has done so at the sacrifice of broader cultural interests. There seems to be general agreement that more men in medicine are needed who can and who are willing to play an important part in public affairs. It was emphasized that while some of the best brains in America are, by a process of selection, being funneled into medicine, these persons are never heard of again in shaping policies in public affairs.

The suggestion was made that the selection of medical students should be done not entirely by medical college officials, but by a team of college officers and medical school officers. It was emphasized that medicine is just as much a social science as it is a biological science. Therefore, it is urgent that the physician have broad training in the social sciences.

With respect to the undergraduate medical school curriculum, general dissatisfaction was expressed. The seminar deplored the attitude of the average medical student that he must pass courses in succession in order that he may reach the stethoscope and scalpel. For example, a famous physiologist stated that, although he had spent his life teaching physiology to medical students, before he dies, he would like to teach the course just once to a group of students who were really interested in the subject. It was pointed out that if the student, for example, could study physiology after he has finished his four years of medicine, he would approach the subject with new interest, enthusiasm and zeal. It was generally agreed that there needed to be closer integration of the various subjects in the undergraduate medical curriculum, although nobody seems to know exactly how to bring them about. There is general agreement that there should be much more emphasis placed now on the problems of psychosomatic medicine, not as a separate course in the medical college curriculum, but that such emphasis should be constantly integrated through all of the courses. Dr. Green, Professor of Philosophy at Yale University, has said that he had been hearing a long time about how medicine had advanced from an art to a science; he thinks it should advance again and become an art, with particular reference to the treatment of psychiatric problems.

There was emphasis on the desirability that the undergraduate curriculum should educate the doctor in his social and moral responsibilities to himself, his patients, his community, and the society in which he lives.

With respect to the training of interns, it is brought out that in the average hospital the rotation system for interns is obviously not for the education of the intern but rather to

cover the various services of the hospital. The educational ideal would be to limit the intern to three services only.

Snapper, of Mount Sinai Hospital, believes that the intern should be made to perform his own laboratory tests and not delegate them to a technician, that he should study the biopsy with the pathologist, and read the x-ray films with the radiologist. Above all, the educational program is a failure if the members of the house staff or the members of the visiting staff regard their daily duties as a chore, to be finished as quickly as possible.

Buerki of Pennsylvania expressed the opinion that the intern should be kept out of the operating room entirely so that he may never presume to think himself capable of doing anything but minor surgery.

Regarding the problems of graduate and postgraduate training in medicine, it is important to remember that graduate training is the long-term experience seen in the residency and fellowship program; whereas, postgraduate training means short courses for the men who are already in practice.

Victor Johnson, of the Mayo Clinic, estimated that we need about 51,000 specialists in this country and stated that we have almost that number now. Any more specialists would be excessive. Although there is agreement that the Specialty Boards in general have fulfilled a very useful function in American medicine, the unusual stress laid upon Board certification has been followed by some unfortunate consequences. For example, there is no excuse for a hospital requiring that a man be a Diplomate of a Specialty Board before he is placed on the staff. The wisdom of a Board of General Practitioners was seriously questioned. If there are 100,000 practitioners in this country and only 10,000 of them become certified, that will leave 90,000 men who obviously have not benefitted from the process at all.

The importance of basic science training at the graduate level was re-emphasized. There was general agreement that a good practitioner cannot be trained with one year's rotating internship. Two years of internship is far better and this training should include general medicine, pediatrics, obstetrics, and only minor surgery.

Middleton, of the University of Wisconsin, expressed the opinion that the chief contribution of the American Board of Internal Medicine has been to provide a sound medical basis for the men such as the allergists, cardiologists, hematologists and chest physicians who practice the medical subspecialities.

No one seems to know exactly how to provide postgraduate training for the general practitioner. The latter will have to be educated in a variety of ways; the method employed will depend upon when and how often he can leave, and more important, if he really wants such training. Buerki stated that medical educators are not keeping the general practitioner up-to-date, and that only about 25 per cent of the practitioners really want to keep up-to-date.

The final discussion in this interesting volume concerns the matter of group practice and the service that such groups can render. In the medical group of the future, the key man will be the one whom we now call the general practitioner. In reality he will be the most important man in the team. He would come in contact with the public more than any others, and he would call upon his colleagues in the specialties to confirm diagnoses and furnish the necessary treatments.

One speaker was much impressed with the experiment now going on of pushing academic medicine into a place of responsibility for the medical care of the people, as illustrated by the veterans' medical program.

Altogether this is a most serious, instructive and informative volume, summarizing well many of the problems confronting medical education today. It indicates that the medical colleges are seriously striving to meet their obligations and responsibilities to society.

Birmingham, Alabama ROY R. KRACKE

George R. Minot Symposium on Hematology. Edited by WILLIAM DAMESHEK, M.D., and F. H. L. TAYLOR, PH.D. 984 pp., illus. $12.00. New York: Grune and Stratton, 1949.

Dr. George R. Minot was 60 years old in 1945. On that occasion a group of his colleagues conceived the idea of honoring him by publication of an Anniversary Volume of writings by his pupils, associates and friends. The manuscripts were first published in Blood, The Journal of Hematology, beginning with January 1948, and continuing through February 1949.

The volume contains 84 articles by 155 authors and coauthors. They have been arranged in six groups of related subjects, dealing with pernicious anemia, hemolytic anemia, anemias other than the two mentioned, blood clotting phenomena and hemorrhagic diseases, leukemia, and biologic and cytologic reactions of blood and blood forming tissues.

Part seven, entitled General Practice, is not in tune with the rest, with its two brief articles on occupational incidence of peptic ulcer and on diabetic coma.

The list of authors reads like Who's Who and the list of subjects like What is What in hematology. All hematologic subjects of any consequence to the practitioner, to the specialist in internal medicine and in hematology, to the clinical pathologist, and to the clinical and experimental investigator are covered by leading authorities. The articles are, without exception, reports on original and significant research, beginning appropriately, with a reprint of Minot and Murphy's *Treatment of Pernicious Anemia by a Special Diet*, from the Journal of the American Medical Association, 1926, the contribution which won them the Nobel Prize in 1934.

It is safe to predict that this book will become a milestone in hematologic literature, a brilliant summation of what is now known and unknown in this rapidly developing field.

Chicago I. DAVIDSOHN

Year Book of Pathology and Clinical Pathology. Pathology, edited by HOWARD T. KARSNER, M.D., Professor of Pathology, Director of the Institute of Pathology, Western Reserve University, Cleveland. Assistant Editor: HERBERT Z. LUND, M.D., Assistant Professor of Pathology, Western Reserve University, Cleveland. Clinical Pathology, edited by ARTHUR HAWLEY SANFORD, M.D., Professor of Clinical Pathology, University of Minnesota (The Mayo Foundation); Senior Consultant, Division of Clinical Laboratories, Mayo Clinic. 538 pp., 127 illustrations, $4.50. Chicago: The Year Book Publishers, Incorporated, 1948.

The 1948 yearbook is organized similarly as its 1947 predecessor. Five special brief reviews have been contributed: Pathogenesis of Shock, by Koletsky; Evaluation of Exfoliative Cytology from Viewpoint of Pathologist, by Foot; Pulmonary Berylliosis, by Dutra; and Iron Metabolism, by Kinney, Hegsted and Finch.

The current interest in exfoliative cytology is attested to by the large amount of space it occupies. In addition to Foot's special contributions in which 5 papers are reviewed, 13 other articles are abstracted in the Chapter on Pathology of Tumors, and five more in the chapter on Cytology, altogether 28 pages, almost 6 per cent of the entire book. Similarly, considerable space is devoted to pneumoconiosis. The special article on iron metabolism refers to 21 articles. Rh factor is represented by 7 articles abstracted on 11 pages.

Every article included in the abstracts will no doubt be of interest to many; it would have been impossible to include all articles of interest to specialists in a volume of this kind. The selection was judicious, and the abstracts are well done. The book is a splendid investment for the pathologist and clinical pathologist.

I. DAVIDSOHN

Mikroskopische Technik. Edition 15. By BENNO ROMEIS, M.D., Professor of Anatomy, Director of the Institute of Histology and Experimental Biology, University of Munich. 695 pp., 21 figs. Munich, Germany: Leibniz Verlag, 1948.

The new 15th edition of Romeis' famous Taschenbuch has outgrown its Taschenbuch format. Otherwise, except for the inclusion of a fair amount of new material, the character and organization of the original work has been retained unchanged. Unlike the favorite American texts, this book is encyclopedic and historical rather than practical. Methods

used by pioneers of normal histology (such as Benda, Golgi, Held and others) which have little interest to the modern investigator and especially to the pathologist, are described in great detail. There is a complete enumeration of Becher's lake-dyes most of which never gained any foothold in histologic technic. On the other hand, new technics of histochemistry are not mentioned. There is no chapter on the staining of bacteria. The majority of the methods are presented without any comment; critical remarks by the author are relatively few.

The value of this book lies in its completeness (up to about 1940) and its excellent bibliography. As a guide to Pathologists and to technicians, it is distinctly inferior to the better American texts.

Chicago GEORGE GOMORI

Blakiston's New Gould Medical Dictionary. First Edition. Edited by HAROLD WELLINGTON JONES, M.D., NORMAND L. HOERR, M.D., and ARTHUR OSOL, PH.D., with the cooperation of an editorial board and 80 contributors. 1294 pp., 252 illustrations, 129 in color. $8.50. Philadelphia and Toronto: The Blakiston Company, 1949.

This new illustrated dictionary is vastly superior to its predecessor which had gone through five editions from 1926 to 1941. The first dictionary by Gould, however, was published in 1890. This dictionary is not a mere revision of an old edition, but rather "an entirely new work". The words are easy to find, easy to read and well defined. The definitions include modern usages. In addition, the work possesses a number of other useful features. This new dictionary gives one a sense of satisfaction in its use. The editors and publishers have apparently spared no effort or expense in its preparation. They are to be highly commended for this excellent contribution to medical science.

Medical Etymology. By O. H. PEPPER, M.D., Professor of Medicine, University of Pennsylvania, Philadelphia. 263 pp. $5.00. Philadelphia: W. B. Saunders Company, 1949.

The author of this concise and useful guide to medical terminology must have been impelled to prepare this work as a logical result of years of medical teaching. Every student of medicine, dentistry and nursing should be required to own or have constant, ready access to this volume. One ventures to suggest that, even after student-days, reference to this volume will be found of value.

There is no question but that its use will make medical terms more meaningful. The book is concerned with the origin of terms rather than with their definition. Special chapters are devoted to common terms used in each of 17 different specialties in medicine, both in preclinical and clinical subjects; a separate chapter is given over to dental terminology. Nearly 4,000 terms are analyzed in one or more sections.

SELECTED ABSTRACTS

The Blood and Bone Marrow in Patients with Cirrhosis of the Liver. L. BERMAN, A. R. AXELROD, T. N. HORAN, S. D. JACOBSON, E. C. VONDERHEIDE AND E. A. SHARP. Blood, 4: 511–533, 1949.

The study is based on a review of the literature and the authors' analysis of 25 patients with diagnoses of cirrhosis of the liver verified by biopsy. Quantitative data and clinical findings are given in tables and appended case reports. The principal blood findings were macrocytic or normocytic anemia with normal or elevated mean corpuscular hemoglobin values, lymphopenia, and thrombocytopenia in the majority of cases. Anemia was independent of bleeding, and the severity of anemia or macrocytosis was not related to the severity or duration of the liver lesion. Absolute lymphopenia, regardless of the total leukocyte count, was the most constant significant alteration of the leukocyte picture.

The consistent change in the bone marrow was extension of the marrow organ so that active hemopoiesis occurred in the shafts of the long bones. Even in patients with advanced liver lesions hypocellularity was an unusual finding in the marrow. Megaloblastic erythropoiesis was not found in any of the patients.

The authors review various theories of the mechanism of the anemia in cirrhosis, and suggest that hemodilution and hemolysis (related to hypersplenism) may be important factors for the low red cell counts seen in many patients with the disease. The combined blood and sternal marrow study was considered useful in establishing the diagnosis of cirrhosis of the liver in patients in whom other diseases have obscured its manifestations or in whom historical evidence was absent so that the clinical diagnosis was difficult.

Detroit LAWRENCE BERMAN

Osmotic Resistance of Erythrocytes from Patients with Carcinoma. T. H. WILSON and W. E. LANDMESSER, JR. Cancer, 2: 503–509, 1949.

A study of the osmotic resistance of red blood cells using 4 different solutions was made on 90 white patients with a variety of malignant diseases, and compared with normal persons. 23 cases of benign gastric and duodenal ulcer were included. Negroes were not included because of an extremely wide range in the normal subject. The solutions used were: (1) 0.18 M glycerol in 0.36% NaCl, (2) 0.24 M thiourea in 0.18% NaCl, (3) 0.47% NaCl, and (4) 0.36% NaCl.

Statistical data on carcinoma of the breast, stomach and colon are given. In the majority of instances the patients with breast carcinoma had red blood cells with normal resistance, while those of the other 2 groups showed increased resistance. The role of anemia and metastasis in the production of this resistance is not made clear. Patients with benign peptic ulcer showed a normal response. The authors are careful to emphasize that these observations are not presented as a "new test" for cancer, since red blood cells from patients with pernicious anemia and the carrier form of Mediterranean anemia also show increased osmotic resistance.

Brooklyn LEO M. MEYER

An Evaluation of Sternal Aspiration As An Aid In Diagnosis of the Malignant Lymphomata. TALBERT COOPER and CHARLES H. WATKINS. Blood, 4: 534–549, 1949.

The authors investigated the sternal marrow of 15 patients with Hodgkin's disease, 10 with lymphosarcoma and 2 with follicular lymphoma. Smears and fixed sections of marrow units were studied. There were no consistent abnormalities or specific changes encountered in the group of Hodgkin's disease, although many patients showed myeloid hyperplasia with shift to the left. The authors suggest that, because of the high incidence of positive findings in postmortem sections taken at random in cases of Hodgkin's disease, multiple aspirations at various sites may prove more fruitful. In 7 of the 10 patients with lympho-

sarcoma there was lymphocytosis of the bone marrow, varying from 30 to 70 per cent. Four different types of abnormal lymphocytes were found. Erythroid elements were reduced. In follicular lymphoma, no diagnostic features were observed.

This reviewer has examined the bone marrow content of many patients with these diseases and has noted several points of difference. In the lymphosarcoma group we have never observed the degree of lymphocytosis of the marrow which was reported, unless a focal area of invasion was aspirated. When the lymphocytes constituted over 25 per cent of all nucleated cells, continued observation of the patients revealed the gradual development of the blood picture of chronic lymphocytic leukemia. Such cases have frequently been mislabelled "leukosarcoma". In the follicular lymphoma group, we have frequently found up to 30 or 40 per cent of extremely small (7–8 mm.) darkly-stained lymphocytes with no rim of cytoplasm in the bone marrow. These cells closely resemble those seen in the peripheral blood and bone marrow of infants, young children and patients with infectious lymphocytosis. These were formerly called "hematogones".

LEO M. MEYER

Heterologous Ocular Transplantation as a Practical Test for Cancer. J. A. SCHILLING, A. C. SNELL, JR., and B. V. FAVATA, Cancer, **2**: 480–489, 1949.

It was the purpose of the authors to determine the practical value of the method in the diagnosis of cancer. Growth occurred in only 8 of 36 cases. Simultaneous tissue cultures of the same tumors grew in 15 of 19 cases. Greater success was noted in the group of connective tissue neoplasms. The limitations of the method were found to be infection, low tumor-content of the transplanted fragment, and desmoplastic reaction. The authors conclude that with the high incidence of failures (78 per cent) in proved cases of malignancy, the method is not of practical value. On the other hand, they do not deny the merit of this procedure as a tool for investigative work, and the opportunity of identifying a tumor which on routine histologic study has been found to be obscure. They also emphasize the observation made by Greene that successful growth of tumors indicates a high degree of autonomy. A concise historical summary of the method dating back to 1873 is included in the paper.

LEO M. MEYER

Heinz Body Phenomenon in Erythrocytes. A Review. S. H. WEBSTER. Blood, **4**: 479–497, 1949.

This review of the Heinz body is complete, and includes historical background, microscopic appearance, staining qualities, chemical nature, mechanism of formation (*in vivo* and *in vitro*), relationship to methemoglobulin, and the role of the spleen. It is strange that so little emphasis has been placed on the significance of these structures, considering their close association with drug and industrial poisonings in man. The drugs most frequently associated are phenyl hydrazine and its acetyl derivative, the sulfonamide groups, and other compounds having a hemolytic action on red blood cells. The damage to erythrocytes is usually followed by the production of these bodies within the cells and, if severe enough, is frequently associated with evidence of hemolysis and anemia. The paper is a comprehensive review of the subject, and includes an extensive bibliography of clinical and experimental data.

LEO M. MEYER

Die akute Silikose des sarganser Beckens. E. UEHLINGER. Schweiz. Ztschr. f. Path. u. Bakt., **12**: 150–157, 1949.

Les Lésions Vasculaires dans le Poumon Silicotique et leurs Relations avec la Tuberculose. J. L. NICOD. Idem, **12**: 157–160, 1949.

At the 14th annual meeting of the Free Association of Swiss Pathologists, held at Zurich, June 19–20, 1948 two papers were presented on silicosis. Uehlinger's contribution concerns acute silicosis encountered recently in Switzerland. Veins of quartz are found

throughout the Sargan Basin area where the Swiss were constructing (1940–1945) fortifications. Of 22 miners who were engaged on the project, by the end of 1947, "12 had died of silicosis and tuberculosis, 9 suffered from silicosis, grades I–III, one had questionable silicotuberculosis". The clinical data are to be found in another publication (Lang, F., and Zollinger, R.: Schweiz. Ztschr. f. Unfallheilk., 1949).

Of the 12 subjects who died, 10 came to necropsy. The time of exposure varied from 10 to 36 months, the latent period (beginning of exposure to invalidism) from 43 to 72 months (median, 57 months), and the lethal interim (beginning of exposure to death) from 69 to 93 months (median, 77 months). Both clinically and radiographically there are two phases; in the first, there is an extensive diffuse pulmonary storage of quartz; in the second or lethal phase, there are massive central masses and peripheral plaques in the middle and lower lobes along the interlobar spaces.

At necropsy the appearance of acute silicosis of miners is uniform. Indurative areas, up to the size of apples, are encountered in the central parts of the lungs and plaque-like scars (1–2 cm. thick) along the lobar borders. The larger lesions, basically olive-green with gray stippling, are composed of smaller nodules, up to the size of a pea. In addition, there is a fine gray-green reticular pattern with submiliary thickenings at the points of intersection; these are largely in the lower lobes. There is pronounced hyperemia and swelling of the tracheobronchial lymph nodes. As in other forms of silicosis, exudative tuberculosis is an added component in 5 of the 11 cases.

Microscopically in the early or first phase, there is deposition of quartz dust along the entire pulmonary skeleton without special storage in the lymphatic apparatus. Silicosis of the lower lobes apparently leads early to respiratory insufficiency. The second phase is initiated by hyalinization of the tissues storing the quartz. The large shadows in acute silicosis, seen roentgenologically, are not produced by confluence of individual foci, but are the result of "massive interstitial and intra-alveolar dust storage with consequent diffuse sclerosis". The tracheobronchial lymph nodes are relatively spared, perhaps because there is insufficient time for massive passage of quartz dust from the lungs or because there is interference within the lung of such passage.

It is concluded that acute silicosis develops when there is exposure to large quantities of quartz dust of fine size (under 3 microns). Both criteria were met in the mining operations where quartz content of some veins is as high as 64 per cent.

Nicod's contribution on "The vascular lesions in the silicotic lung and their relation to tuberculosis" is based on the study of lungs from 27 miners with silicosis. The vascular changes, superficially quite varied, are, in reality, limited. Everywhere, especially in the less diseased lungs, the adventitia is converted into a histiocytic cuff with the offending dust in the cells. Sometimes the intima escapes, but often there is eccentric fibrous thickening, with dust-sprinkled cells among the collagen bundles. Since such cells are not encountered in the media, non-adventitial origin is not unlikely. Nicod suggests that the endothelium may fix silicic grains assumed to be in the circulating blood. Medial changes are largely confined to disappearance of elastic fibres. The process, associated with the intimal disease, starts at the medio-adventitial junction and proceed toward the internal elastic membrane. True arteritic or phlebitic stigmata are not recognized.

The vascular lesions are not unique to silicosis, but there is "a certain air of specificity" in that an altered vessel may lie next to a virtually normal one. Similar changes are found in the lungs of tuberculous miners, but always with additional features of tuberculous vascular contamination.

Tuberculosis (Ranke II type) is frequent among pneumonoconiotics. Nicod records 6 per cent of hematogenous lesions among 142 silicotics. He also points out that the tuberculous lesions are of the same appearance and importance whether or not dissemination is by the vascular route. He remarks that the explanation of the frequency of tuberculosis in silicotic patients is not to be found in simple morphology.

Discussing Nicod's presentation, Roulet offers the concept of "microsilicosis". In older people, there is, with increasing age, increasing content of silicates at the pulmonary api-

ces. Under these circumstances, deposition dust in the tracheobronchial lymph nodes is often profound, especially in the presence of chronic (deforming) bronchitis, which Roulet links to this type of "silent" silicosis.

Fort Wayne S. M. RABSON

Die Wirkung verschiedener Kieselsäureformen im sensibilisierten Tierorganismus. WILHELM KOCH. Frankf. Ztschr. f. Path., 60: 58–96, 1949.

Koch introduces his contribution on "The action of various forms of silicic acid in the sensitized animal organism" with a review of the present status of our knowledge of the fate and effects of the silicates in animals and man. He presents four anatomic sites in the respiratory tract as deserving consideration: nose and nasopharynx; larynx, trachea and bronchi; the total alveolar apparatus; pulmonary connective tissue network and lymph channels. Koch's experiments are devoted to the last of these four sites. The subcutaneous route alone was used for injection of the rabbits. Quartz dust of varied fineness, colloidal silicic acid solution, and silicic acid with aerosol properties were among the six substances employed. The animals were sensitized to dried egg-albumen, nucleic acid (fecal), and an antigen mixture of pneumococcus culture-filtrates and organ-extracts. Injections were given over different periods, and most animals were examined as late as three years after the beginning of the experiment.

In a relatively short time "typical" silicotic nodules developed in the subcutis, and it was possible to follow the anatomic changes from foreign body granuloma, "resorption" granuloma, and necrosis-to-fiber formation. In two animals, 1½ years after injection with similar doses of silicic acid, subcutaneous cysts ("Breicyste") were formed. In none of the silicic acid (colloidal; non-colloidal) experiments did anything more than a few scattered nodules appear, and the cysts did not develop at the site of injection.

Koch also emphasizes the role of fatty substances and of iron during the phase of the "resorption" granuloma produced by both silicic acid series. He suggests that the detoxification of soluble silicic acid may be accomplished by esterification with cholesterol and phosphatids. It may also take place by the introduction of molecules of silicic acid into the fatty acids or their esters, followed by the formation of a transportable form when protein colloid is added. Esterification is accelerated by the presence of local oxygen or of oxygen-carrying ferments.

Part of the effect of quartz depends on its solution in the tissues. There is also a mechanical effect which begins immediately after injection of quartz into the subcutis, which then becomes incorporated into the post-solution phenomenon. That the result is partly mechanical is demonstrated by the absence of connective tissue proliferation when colloidal silicic acid is used. Koch also considers another factor, the electrical charge of the particles of crystalline silicic acid.

Sensitization to foreign protein displaces the reaction to colloidal silicic acid in the direction of increasing tolerance of the acid in smaller and medium doses, and in the direction of heightened tendency to necrosis in medium and greater doses. As far as tissue response to quartz injections is concerned, it is considered probable that the sensitization strengthens the necrotic tendency and speeds up the formation of fibrous tissue. Koch believes that severe local damage by the continual flow of quartz particles of colloidal silicic acid is present only when the reticulo-endothelial apparatus, which is attempting to detoxify, becomes "paralyzed". Silicotic scars and the silicotic cyst ("Breicyst") are regarded as the expression of the local increase of concentration of quartz or of colloidal silicic acid. It is considered that the change in the action of these two substances by foreign protein sensitization is a "parallergic" phenomenon, and in part merely the simple summation of stimuli.

The action of the air passages in filtering dusts influences the amount of dust and the size of the particles which reach the pulmonary connective tissue network.

The tendency to the development of severe dust disease in the lungs rests on the individual response to the dusts deposited in the network and in the lymph nodal reticulum.

The tendency is influenced by the available reactive lipoids, the efficiency of protein metabolism in supplying oxidative ferments, and the tonus of the vegetative nervous system in the area of the lung segments. Koch concludes that the processes described in his paper may shed light on the action of other toxic agents, on the pathogenesis of tuberculosis, and on the relationship of silicosis and tuberculosis.

S. M. RABSON

Brucellotic Osteomyelitis of Man and Animal. LEO LOWBEER. Proc. Staff Meet., Hillcrest Mem. Hosp., Tulsa, **6:** 1–36, 1949.

Chronic disease remains one of the major handicaps to achieving a happier middle and old age. Of the chronic ailments brucellosis is assuming even greater importance as its pathologic anatomy and immunology are investigated. Among the relatively few workers devoting major attention to this malady is Lowbeer. It is unfortunate that the results of his studies hitherto have been published in local journals, and it is to be hoped that in the future other facilities will be used to reach a larger medical public. The paper here reviewed was presented before the recent Inter-American Congress on Brucellosis held in Buenos Aires, November, 1948.

The introduction consists of a brief review of the pathogenesis of the brucellotic state, especially in the osseous tissues. Major attention is given to the much truncated findings in 4 human, and in 4 porcine cases of brucellar osteomyelitis, together with summaries of clinical findings.

The anatomic results made it possible to trace the development of the disease through its several phases. The early stage differs from pyogenic osteomyelitis by being less purulent and extensive locally and by a greater tendency to repair. Later, the granulomatous character is well pronounced with central necrosis and peripheral fibrosis and growth of new bone. Tissue destruction is "apparently rapid but self-limited . . . (and) soon becomes localized". There were no significant differences in the nature of the brucellotic granuloma in man and in animals.

There are 61 accompanying illustrations, of gross and microscopic preparations as well as of roentgenographic films. Size and quality of reproduction leave much to be desired, especially of photomicrographs. The extensive bibliography of more than 200 publications is numbered, but fewer than a dozen of them are referred to in the text. These deficiencies in this worthwhile project should be corrected in future publications.

S. M. RABSON

A Study of Certain Aspects of Blood Coagulation in the Postoperative State in Congestive Heart Failure and in Thrombophlebitis. NOBLE O. FOWLER. J. Clin. Investigation, **28:** 671–678, 1949.

The study was made to determine if there is any correlation among the various tests used to detect increased coagulability of the blood. These procedures are coagulation time (Lee-White, lusteroid tube, silicone tube), clot retraction, Waugh and Ruddick heparin test, heparin tolerance test, and estimation of the prothrombin time employing both whole and diluted (12.5 per cent) plasma. An attempt was made to determine if any of the tests can be used to predict venous thrombosis in patients. In addition, the frequency of hypercoagulable blood in the postoperative state and in congestive heart failure was studied, and observations were made on the effect of tube size and temperature on the Lee-White coagulation time.

Twenty-nine patients were studied postoperatively, as well as 27 patients with congestive failure, three patients with acute thrombophlebitis, and a group of normal controls. Various modifications of the technics were used to increase the sensitivity of the procedure. Prothrombin estimations were made by the Quick technic using Squibb's thromboplastin.

The author found poor correlation among the various tests used to detect increased coagulability of the blood. This suggests that the procedures are dependent upon different

factors in the clotting mechanism. He also found hypercoagulability of the blood frequently in the postoperative period. It was detectable as long as 12 days after surgery. It was not possible to select the candidates for venous thrombosis by changes in the coagulability of the blood. The author believes the Waugh-Ruddick test to be more sensitive than the others, as used in this study, as an index of blood coagulability. Its lack of specificity, it is believed, robs it of its value in predicting or diagnosing venous thrombosis.

New York SHEPARD SHAPIRO

The One-Stage and Two-Stage Prothrombin Methods in the Control of Dicumarol Therapy, with Remarks on AC Globulin. JOHN H. OLWIN, M.D. J. Lab. and Clin. Med., 34: 806–813, 1949.

A study of the comparative results of prothrombin estimation obtained by the one and two-stage methods. The experience with the behavior of accelerator globulin is reported and the possible relationship to disparities between the two methods of estimation of prothrombin time is discussed.

One hundred and forty patients receiving Dicumarol were studied. In the first 99 the two-stage method was used; in the next 41, AC globulin was also tested, according to Seegers. In 95 of the first 99 patients, the Quick method was run parallel with the two-stage method. In 10 of the first 99 cases the Quick method was employed using 12.5 per cent plasma and 5 per cent plasma.

Initial doses of Dicumarol of 200 to 500 mg. were given, followed on the next day by 100–200 mg. and then by variable doses depending upon the prothrombin time. After the patients were "stabilized", prothrombin estimations were made at weekly intervals and in some cases every two weeks. Periods of medication varied, the average being 60 days, the maximum 2 years.

The two-stage method was found more accurate and safer than the single-stage procedure for control of Dicumarol medication. The accelerator factor was depressed initially after Dicumarol but returned to normal after 21 days. The reasons for the disparity between the two methods of prothrombin estimation were not uncovered in the study.

The normal and therapeutic range of prothrombin time are not stated. With the one exception of a chart containing diluted plasma prothrombin times, no time values are given, and percentages are reported exclusively.

Estimation of prothrombin time of diluted plasma was made in only 10 cases. Five per cent plasma was employed but it is not stated if the end-point was satisfactory, especially after Dicumarol.

SHEPARD SHAPIRO

Blood Histamine in Leukemia and Erythremia. M. B. SHIMKIN, L. SAPIRSTEIN, F. R. GOETZEL, P. M. WHEELER, AND N. I. BERLIN. J. Nat. Cancer Inst., 9: 379–387, 1949.

Over a decade ago the finding of increased quantities of histamine in the blood of patients with myelocytic leukemia was reported. The present report concerns itself with a series of patients with various leukocytoses, myelocytic and lymphocytic leukemias, and erythremia, who were studied with regard to the histamine content of their whole blood and plasma.

Normal histamine values in the blood of adults fell between 1.8 and 8.5 micrograms per 100 ml., with an average of 4.0 micrograms. These values have been found to be almost identical on repetition at a later date. Previous work has localized 80 per cent of the blood histamine in man in the white blood cells, chiefly in the granulocytes. Slight traces are present in the plasma. The histamine content, however, is not directly related to the number of circulating granulocytes, as shown by the relatively normal values in simple leukocytoses and eosinophilias.

Marked increases occurred in myelocytic leukemia (in one patient being as high as 1680 micrograms per 100 ml.) in the presence of a substantial increase in the leukocyte count.

The levels were higher in chronic myelocytic leukemia than in acute myelosis or acute exacerbations of chronic myelosis. The blood histamine levels were essentially normal in lymphocytic and monocytic leukemias, and slightly elevated in erythremia.

New Orleans, Louisiana BEN FISHER

The Nature of Russell Bodies and Kurloff Bodies. Observations on the cytochemistry of plasma-cells and reticulum cells. A. G. PEARSE, J. Clin. Path., **2**: 81, 1949.

This interesting paper will be welcomed by many pathologists who have long been curious as to the nature of the often-seen Russell bodies. The Kurloff bodies, found in guinea-pig splenic and blood lymphocytes, are thought to be related structures.

The author studied both types of bodies by specific histochemical methods before and after treatment with ribonuclease, hyaluronidase, and diastase, and gives his conclusions as to chemical composition of each. He discusses the significance of the findings in both bodies of mucoprotein and ribonucleic acid in relation to the various theories of their formation.

The evidence presented points to the probability that the mucoprotein is secreted by the parent cell rather than that it is stored or absorbed from without. He lists in detail the technics used and presents photomicrographs and an extensive bibliography.

Indianapolis, Indiana C. G. CULBERTSON

The Microbiological Assay of Riboflavine and Nicotinic Acid in Urine. J. FITZPATRICK AND S. L. TOMPSETT. J. Clin. Path., **2**: 121, 1949.

This article describes the assay of the substances mentioned in the urine, using *Lactobacillus helveticus* (casei) N.C.T.C. No. 6375 for riboflavin and *Lactobacillus arabinosus* 17/5 N.C.T.C. No. 6376 for nicotinic acid.

Instructions for preparation of the synthetic medium used are given. Details concerning collection and dilution of urine are stated. Studies on the recovery of the substances after adding definite amounts to urine specimens are reported for five different cases.

C. G. CULBERTSON

Rapid Determination of the Sickle Cell Trait by the Use of a Reducing Agent. A. W. WILLIAMS AND J. P. MACKEY. J. Clin. Path., **2**: 141, 1949.

After discussing previously-reported methods for the determination of the presence or absence of the sickle cell trait, the authors describe their own method using sodium hydrosulphite. The method yields results in 20 minutes comparable to the sealed wet preparation read at 48 hours. The results of these methods are in good agreement.

C. G. CULBERTSON

The Determination of the Total Neutral 17-Ketosteroids in Urine. S. L. TOMPSETT. J. Clin. Path., **2**: 126, 1949.

The author describes a method which modifies previously reported methods using acid hydrolysis and ether extraction and colorimetric estimation by the Zimmermann reaction. When applied to urinary extracts there is an interfering brown, nonspecific coloration. The author, therefore, suggests the use of Girard's reagent T. This does not completely eliminate the brown coloration but directions are given for dealing with it by use of filters.

The methods are listed in detail and a comparison of results obtained by the two methods of extraction is given.

C. G. CULBERTSON

Elution of an Incomplete Type of Antibody from the Erythrocytes in Acquired Hemolytic Anemia. P. KIDD. J. Clin. Path., **2**: 103, 1949.

The author cites evidence from the literature that it is possible to differentiate between congenital and acquired types of acholuric jaundice by the Coombs anti-human globulin

serum technic. The antiserum agglutinates the erythrocytes of patients with acquired hemolytic anemia but not of those with anemia of the congenital variety. In studying six cases of the acquired type of disease the author confirmed this observation and tested for the "incomplete" antibody of Race, which he found to be negative in the serum of these individuals. He reports a technic for elution of the "incomplete" antibody and reports studies on the properties of this antibody.

All technics are described in detail and the resulting eluted antibody was studied as to titer, range of erythrocytes affected, and thermal amplitude, as well as its effect on the fragility of normal erythrocytes. All human red cells can be sensitized by the eluates without regard to usual or Rh grouping. Titers ran from 1:16 to 1:256. When the thermal amplitude was set between 40 C. and 37 C. no effect of changes in temperature between these limits was noted. Eluates did not significantly alter the osmotic fragility of normal red cells nor of those from two patients with the disease in the series.

Experiments to demonstrate the presence of a "blocking" effect to cold agglutinins, present in some cases of acquired hemolytic anemia, failed to demonstrate this effect.

Discussion of the significance and many other details of the findings are given.

C. G. CULBERTSON

The Iron Reserve of a Normal Man. M. HYNES. J. Clin. Path., **2:** 99, 1949.

The author reports studies designed to determine the iron reserve by bleeding the individual until the rate of hemoglobin regeneration falls to a constant level. The daily absorption from the food in excess of the normal requirement to replace worn-out red cells was calculated from the different rates of hemoglobin regeneration when 6.5 and 20 ml. of blood were withdrawn daily after exhaustion of the iron reserve. He calculates the iron reserve value at 600 mg.

The author states that the iron reserve "would not be depleted by blood donations of 450 ml. as often as five times yearly by a man or by a woman with normal menstrual loss." He states that the average English diet can yield 4 mg. of iron daily for hemoglobin synthesis above the normal needs.

C. G. CULBERTSON

The Surgical Pathology of Rectal Cancer. C. E. DUKES. J. Clin. Path., **2:** 95, 1949.

Dr. Dukes presents statistics from an analysis of 716 cases treated by radical excision between 1928 and 1941. All but 12 patients were followed for a period of five years.

The author classifies the tumors into three main groups, adeno, colloid, and simplex, which grouping he believes to be significant in relation to prognosis. Definition and description of each variety is presented in considerable detail.

He stresses the importance of and presents methods for determining the extent of local spread. Venous and lymphatic spread are discussed.

In this series 50 per cent of patients were living at the end of five years.

C. G. CULBERTSON

Cystic Pneumatosis of the Large Intestine. I. FRIEDMANN. J. Clin. Path., **2:** 91, 1949.

The condition reported is defined as the presence of gas in cyst-like formations in the gastrointestinal tract. The literature reviewed states some 200 cases have been reported, predominantly in adults. The causes as well as the gross and microscopic pathology are discussed. The condition is difficult to diagnose clinically and has usually been recognized at operation or at necropsy and more recently by X-ray.

A case history is presented along with photomicrographs and a bibliography.

C. G. CULBERTSON

Orthotolidine Hydrochloride Test for Blood in Urine. H. ZWARENSTEIN. J. Clin. Path., **2:** 145, 1949.

A simple method for determination of blood in the urine is described. The correlation

between the results of the test and the amount of hemoglobin present, as well as the substances giving false positive tests, are mentioned.

The sensitivity of the test is said to be greater than the microscopic test for red cells and sixteen times more sensitive than the spectroscopic test for free hemoglobulin. The method is said to be able to detect two red cells per cu. mm. and as little as 150 micrograms hemoglobin per 100 ml.

C. G. CULBERTSON

Opalescence of Serum after Total Body X-Irradiation as a Prognostic Sign of Death. R. L. ROSENTHAL. Science, 110: 43–44, 1949.

Marked opalescence was noted in the serum and plasma of rabbits which died a few days after exposure to a single lethal dose of total body irradiation. Opalescence appeared within 24 hours following radiation and disappeared completely 72 hours after exposure. Rabbits of the New Zealand white strain were given single doses of 200 Kv/X-irradiation over the total body. All animals showing marked opalescence died as a result of radiation within five days following exposure. At present there is no clear explanation of the mechanism. The phenomenon may not only provide a valuable early measurement of the effect of acute exposure to radiation, but may lead to further knowledge concerning the nature of adiation sickness and its lethal mechanisms.

TECHNICAL SECTION

AUTOMATIC TISSUE DEHYDRATING AND EMBEDDING APPARATUS*

JOSEPH TANNENBERG, M.D.

From the Genesee County Laboratory, Batavia, New York

The pitfalls of the present embedding methods became particularly apparent at the beginning of the war when skilled technicians had to be replaced by untrained personnel. Manual transfer of many specimens through several containers filled with various dehydrating and clearing fluids, was time-consuming and not infrequently resulted in confusing the identity of the specimens. The method here described eliminates these risks. The method was developed over a period of eight years and was tested in its final form for over a year. It utilizes a special tissue container and an automatic dehydrating apparatus. The tissue specimens are dehydrated within the same container that is used to form the tissue paraffin-blocks.

THE TISSUE CONTAINER

The tissue container has the appearance of a metal box measuring about 13.2 x 4.9 cm. and 3.5 cm. in height. It is open at the top and consists of six interlocking "tissue carriers proper", a pair of outer sidewalls and a pair of clamps. The tissue "carrier" is the basic unit. It is U-shaped, has a flat bottom and measures 4.7 x 2 cm. The two upright sidewalls have a height of approximately 3 cm. and a length of 4.7 cm. One sidewall carries an interlocking device at its upper end as well as a receptacle for a specimen marker (Fig. 1). This wall should be 1 or 2 mm. higher than the other. Any number of carriers can be interlocked for simultaneous handling, but it has been found practical to unite six of them to form the compartments of one container. The open sides of this interlocked unit are closed by the "outer sidewalls" of the tissue container. The edges of these sidewalls are bent rectangularly to support the bottom and the small sidewalls of the carrier unit. The carrier unit and the sidewalls of the container are held together by one or two U-shaped clamps. Each clamp carries a stout handle on its upper rolled-in edge (Fig. 2). The outer sidewalls of the container and the walls of the carriers, forming one unit, must fit so closely that even minute tissue particles cannot escape from one compartment into another. On the other hand, the dehydrating fluid and melted paraffin must be permitted to enter the compartments from below and through the capillary space between the walls of the compartments. Each compartment can be used for two speci-

* Received for publication, June 10, 1949.

Presented at the Twenty-Eighth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1949.

mens of a size ordinarily used for tissue blocks. If particularly large specimens are to be embedded, larger tissue carriers may be used, one larger carrier replacing two of ordinary size.

After testing various materials, monel sheet metal was found to be the most satisfactory. This had the necessary resistance to corrosion, rigidity and resiliency, as well as the property of releasing easily the paraffin blocks with smooth and even surfaces.

Figure 3 also illustrates a useful variety of a tissue container. In this case the side and end walls are connected by piano hinges with a solid or perforated bottom that has the width of the compartment. We have had good results with this device, but prefer the first model because its construction is simpler and less expensive.

THE AUTOMATIC DEHYDRATING APPARATUS

The automatic dehydrating apparatus is shown in Figure 4, and consists of four parts: (A) a storage tank, (B) a dehydrating tank proper, (C) a collecting tank, and (D) an electric pump.

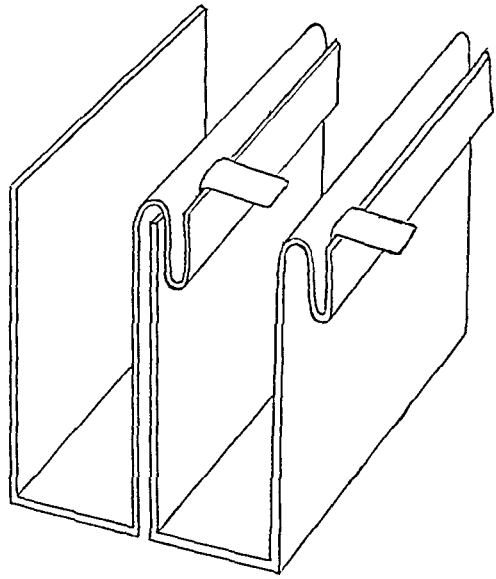
A. A glass bottle holding about 8 liters is used as a *storage tank*. It has an outlet regulated by a faucet a few centimeters above its bottom. Its mouth is tightly closed, either by a rubber stopper protected by metal foil or by a cork stopper protected by paraffin, or by a screw cap or a glass lid with mercury seal. The stopper, or other closing device, is perforated by a metal tube, permitting the inflow of the dehydrating fluid and by a thin vent preventing excess pressure within the tank. The bottom of the tank is covered by a layer, about 1 cm. in height, of calcium chloride or calcium oxide.

Metal containers are not as satisfactory as glass containers. The storage tank is permanently placed above the dehydrating tank to facilitate the flow by gravity of the dehydrating fluid into the latter.

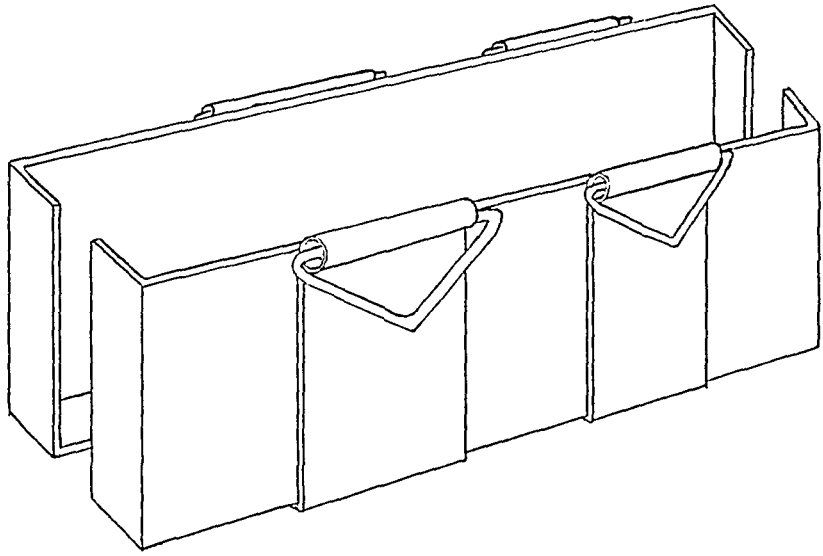
B. *The dehydrating tank* is a metal or glass container about 12 cm. deep and adequate in size to hold 8 to 10 tissue containers. It is connected with the storage tank (A) by a metal inflow tube and, at the opposite side, with the collecting tank (C) by a syphon-controlled outflow. The outflow tube has a larger diameter than the inflow tube in order to permit rapid removal of the dehydrating fluid from time to time while inflow continues at a slower rate. The syphon is so arranged that the outflow begins automatically when the rising fluid reaches a level 2 to 3 mm. below the upper edge of the tissue carriers, and is interrupted when it reaches a low level of about 3 mm. above the bottom of the tissue carriers. This arrangement prevents drying out of the tissues under any condition and makes a lid on the tissue carriers unnecessary. A heavy well-fitted lid makes the dehydrating tank practically vapor-proof. In order to remove water that has been leeched from the tissues as quickly as possible from the dehydrating fluid, glass trays about 4 to 5 cm. deep, containing a layer of 1 to 2 cm. of calcium chloride or oxide, are placed at the bottom of the tank. They are covered by a rack that supports the tissue containers.

C. *The collecting tank* is at least one-third larger than the storage tank and is

also preferably made of glass. It is placed at a lower level than the dehydrating tank (B), to permit the flow of fluid by gravity. Its stopper or lid is perforated



1



2

FIG. 1. (upper) Two "tissue carriers" with interlocking device and receptacle for specimen marker

FIG. 2. (lower) Tissue container consisting of a pair of rectangular sidewalls with bent edges for support of the bottom and small side walls of a unit of 6 tissue carriers

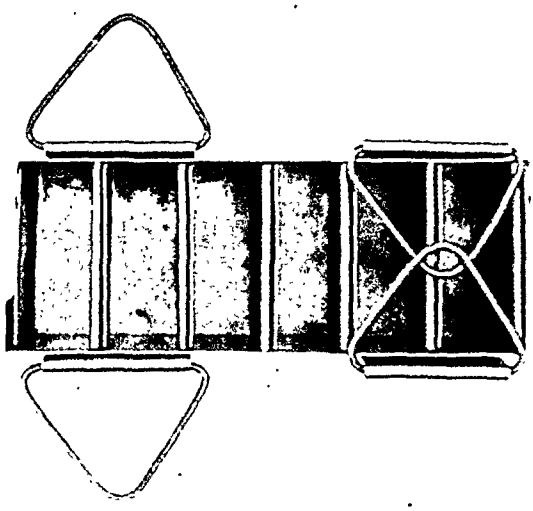
by the outflow tube of the dehydrating tank and by another metal tube for the retransfer of the dehydrating fluid to the storage tank. This is accomplished by an electric pump. A thin vent also perforates the stopper. The bottom of this

tank is covered by a layer 4 to 5 cm. of calcium chloride or oxide. In order to insure that only fluid well-freed of water leeched from the tissues reaches the storage tank to be used again, the transferring tube ends well above the bottom of the collecting tank.

D. *A small truck fuel-pump* operated by direct current from an inexpensive dry battery is adequate for the transfer of the dehydrating fluid from the collecting to the storage tank for re-use. Originally, interchangeable bottles of the same size and with the same fittings were used as storage and collecting tanks. They were simply exchanged after each run. The pumping device was installed particularly to avoid the danger, when handling, of breaking a large bottle containing about two gallons of dioxane.

THE DEHYDRATING AND EMBEDDING PROCESS

Dehydration is accomplished by a process similar to washing out of salt compounds in ultrafiltration, *i.e.*, by moving constantly a large amount of fluid that, in our case, leeches water from the tissues that are kept at rest. The tissue containers with a maximal load for a single run of about 100 specimens are placed at the same level on a rack in the upper half of the dehydrating tank. Its size in relation to the maximal number of specimens handled simultaneously, does not differ materially from that of a container commonly used at present for ordinary dehydration with dioxane. It also contains calcium chloride or oxide to maintain the proper dehydrating quality of the upper layers of the dioxane which actually come in contact with the tissues. In the ordinary dehydrating process using dioxane, 3 or 4 containers are filled with this agent and the tissues must be transferred from one container to another. If the specimens have a thickness of about 2 mm., they must remain 1½ to 2 hours in each container to insure proper dehydration. In our apparatus the dehydrating process is considerably speeded up by the automatic continuous movement of the dehydrating fluid which is present in immediate contact with the specimens, and penetrates them. This is accomplished by a slow but uninterrupted inflow of dioxane into the dehydrating tank, and its *continuous rise from below* to a level of about 2 cm. above the tissues. At this level, just about 2 mm. below the upper edge of the tissue carriers, the rapid siphoning off of the dehydrating fluid begins and lowers the fluid level to that of the tissues, about 3 mm. above the bottom of the tissue carriers. The siphoning effect is then automatically broken and the dehydrating fluid begins automatically to rise again. This arrangement prevents overflowing from one compartment to another of specimens that are eventually floating, and yet does not require a lid for the tissue containers. It also eliminates accidental drying out of tissue specimens, if by chance the flow of the dehydrating fluid should be interrupted. If the inflow faucet is arranged so that the dioxane enters the dehydrating tank at the rate of one gallon per hour, a full load of specimens of ordinary size (each specimen having a thickness of about 2 mm.) may be dehydrated satisfactorily in two hours. During that period at least 5 or 6 changes of the dehydrating fluid will have taken place. Curettings and similar tissue are well dehydrated within one hour. If the dehydration of thicker tissue blocks is



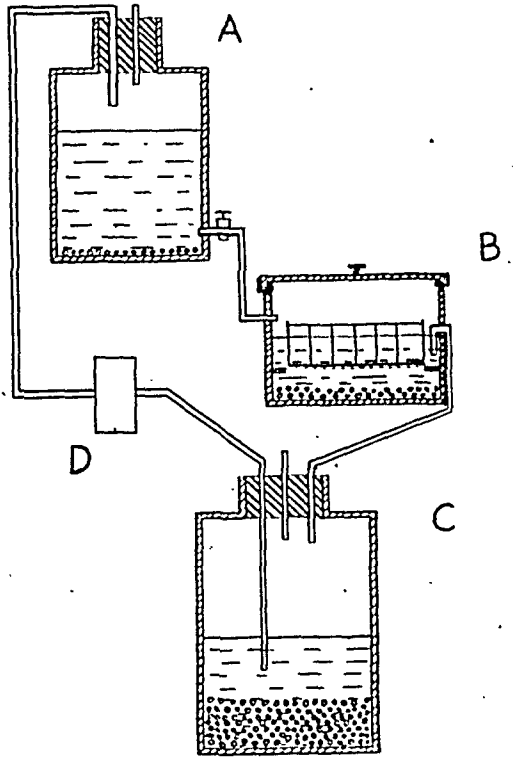


FIG. 3 (upper). Photograph of tissue container with 6 interlocking tissue carriers forming the compartments

FIG. 4 (lower). Diagram of the dehydrating apparatus. A, storage tank; B, dehydrating tank proper; C, collecting tank; D, electrical pump. Note small vents in stopper of each tank.

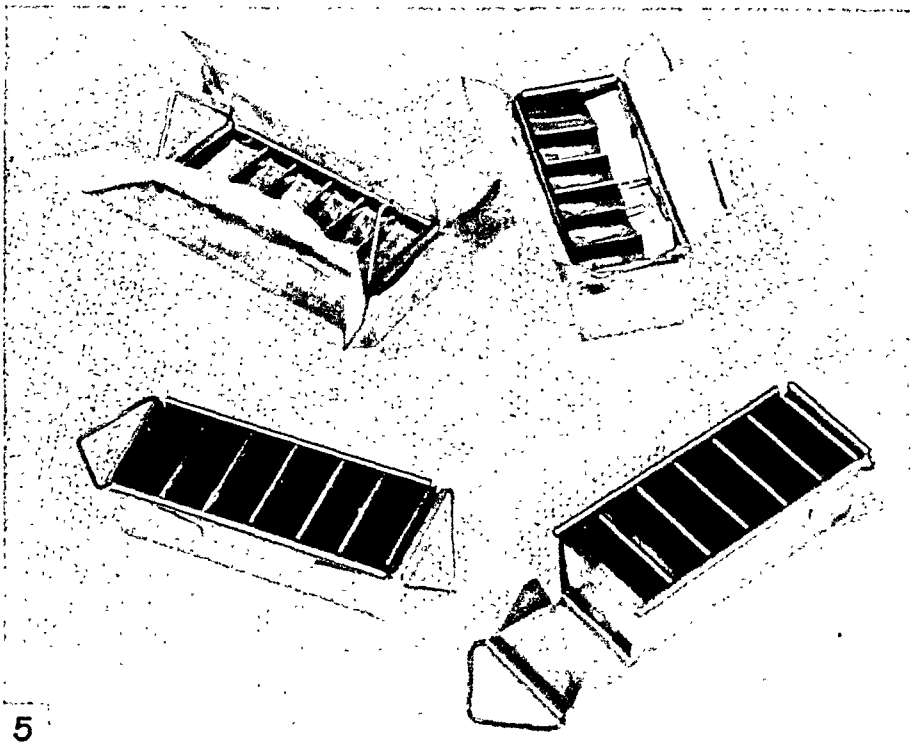
desired, as in autopsy material, particularly of brain or smooth muscle, a longer dehydrating period, preferably overnight, is advisable.

At the end of one dehydrating run, the used dioxane remains in the collecting tank, which contains a deep layer of calcium chloride or oxide until another batch of tissue is to be dehydrated. Then the upper two-thirds of the fluid in the collecting tank is pumped into the storage tank where a thin layer of calcium chloride or oxide is maintained. It serves as an indicator for the condition of the dioxane, rather than for the purpose of freeing water from the dioxane. At a glance at the collecting or storage tank, it can be determined whether or not the dioxane is fit for re-use. We have found that, even if a part of the calcium chloride has gone into solution by water extracted from the dioxane, the dioxane that stands above this solution and does not mix with it has still enough power to dehydrate tissue specimens. We replace the calcium chloride when part of it has become liquefied and add more dioxane at that time.

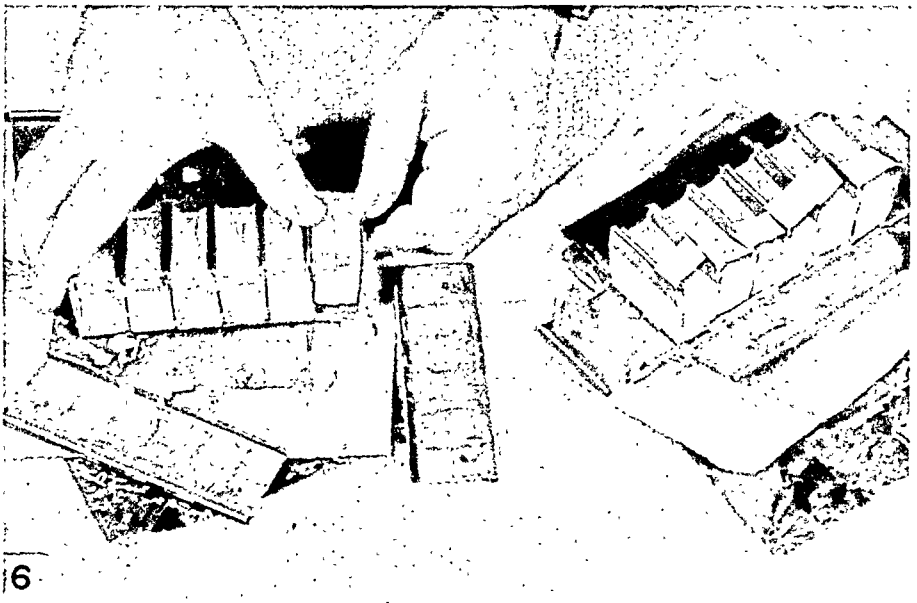
For the past eight years we have been routinely using dioxane for tissue dehydration with excellent results. Dioxane is also successful in the automatic dehydrating apparatus. Acetone also may be used. Alcohol, however, is unsuitable because a succession of alcohols of increasing concentration is required for dehydration. Furthermore, complete removal of the alcohol from the tissue specimen is necessary since alcohol is not soluble in paraffin.

Since the introduction of dioxane (diethylene dioxide) by Graupner and Weisberger,[7] warnings as to its toxicity and ill-effects on staining have repeatedly been voiced. Barber[3] reviewed 5 cases of acute dioxane poisoning. Von Oettingen and Jirouch[17] found dioxane four times less toxic than ethylene glycol in their animal experiments. Fairley,[5] De Navasques,[4] Schrenk,[15] Garvin,[6] Nelson,[12] and others who used dioxane regularly, minimized its danger, if it is kept, like other volatile substances, in well-closed containers. The arrangement of our dehydrating apparatus practically prohibits any escape of fumes into the working room. In this connection the mercury-seal described by Nelson[13] for wide-mouthed jars is interesting. Vandegrift[16] finds dioxane to have an unfavorable effect on acid stains. However, our results with eosin and phloxine stains have been quite satisfactory in routine pathologic material, and we are in accord with Garvin, Nelson, Baird,[1, 2] McClung,[10] and Mettler[11] that dioxane causes little interference with staining.

Following dehydration the dioxane is drained from the bottom and the tissue containers are then immediately transferred to the paraffin oven. We found it convenient to keep square pyrex glass trays about 20 cm. in length and 4 to 5 cm. in depth filled with melted paraffin to a depth of 2 to 2.5 cm., on the shelves of our paraffin oven. We use two changes of paraffin (melting point about 58 C.), allowing one-half to one hour for each paraffin bath. At the end of the impregnation period, the tissue container is lifted by its handles from the paraffin bath and allowed to drain. It is then placed on a sheet of metal (lead) foil that is covered by paper for protection. The foil is folded around the container that is filled again with fresh paraffin to a height of 2 to 2.5 cm. After final arrangement of the specimens with a hot needle, the container with its metal foil



5



6

FIG. 5. Tissue containers, the upper two filled with paraffin and wrapped with metal-foil
FIG. 6. Illustration of simple removal of the tissue blocks from the carriers

wrapping is placed in water at 10 to 12 C. or into the refrigerator as soon as solidification has begun. The metal foil wrapping is particularly useful in preventing entrance of the cooling water into the still soft paraffin blocks. Following complete solidification of the paraffin, the metal foil is easily removed, the clamps which hold the container together are removed by using their handles as levers, and the walls of the container are detached simply by slight outward pressure on their upper edges. Figure 6 illustrates the simple way in which successive paraffin tissue blocks can be removed by hand from the carriers. The block may be trimmed to convenient size or divided into two or three parts by means of a mechanical tissue trimmer, described by Conger in the following article in this issue. To cleanse the tissue carriers of adherent paraffin after use, an inexpensive roaster with a tray covered by a rack was found to be practical. It may be used with hot water or dry heat. A final dip of the parts in used xylol will remove the last adherent paraffin film.

DISCUSSION

The purpose of tissue dehydration is to make tissue specimens suitable for paraffin impregnation. It would be interesting to ascertain the optimal amount of tissue water, which varies from 30 per cent in fat tissue to 80 per cent in muscular tissue, that should be removed. The apparatus described brings about the necessary dehydration in record time for the following reasons: First, a large quantity of dehydrating fluid is used for absorption of a small amount of water. Even a full tissue load of about 100 specimens does not contain more than 70 ml. of water. For its absorption, more than 10,000 ml. of dehydrating fluid is available and kept moving. Second, the slow but continual rise of the dehydrating fluid from the bottom of the dehydrating tank constantly removes whatever water may be present in the vicinity of the tissues and thereby accelerates leeching of water from the specimens. Third, that portion of the dehydrating fluid which has absorbed some water from the specimens, is periodically and quickly removed from the dehydrating tank by the syphon or freed of water by calcium chloride kept at the bottom of the tank. This brings continually fresh dehydrating fluid in contact with the specimens. Since all tissue specimens are placed at the same level and the amount of dehydrating fluid is abundant in relation even to a maximal load, it does not make any difference whether one or 100 specimens are treated at a time. Our method reduces the time required for routine dehydration and paraffin impregnation to about five hours. The paraffin impregnation period, if desired, may be shortened by a vacuum method (Hoffmann 1884).[8] Lilly[9] and Pasternack[11] have in recent years described devices for this purpose.

Our dehydrating method is inexpensive since the apparatus is of simple construction, and the dehydrating fluid can be used over and over again. Only the calcium chloride or oxide, particularly in the collecting tank, must be renewed from time to time. This should be done when a distinct layer of liquid that does not mix with the supernatant dioxane has accumulated at the bottom and reaches a few millimeters above the partly-broken down lumps of $CaCl_2$. Addi-

tion of a bit of cobaltous sulfate stains this layer of oversaturated solution of $CaCl_2$ deep green, while the supernatant dioxane is only faintly stained. To test further the dioxane transferred to the storage tank for re-use, a few granules of soluble anhydrous calcium sulfate (commercially available as Drierite*) can be kept on top of the thin layer of $CaCl_2$ that is maintained at the bottom of this tank. As long as the granules keep their shape and retain some color, the dioxane is practically free of water.

SUMMARY

An inexpensive, automatic, dehydrating and embedding apparatus is described that shortens the dehydrating period considerably, requires no handling of single tissue specimens, and delivers the specimens as paraffin tissue blocks within the same compartments of the tissue carriers in which they were placed prior to dehydration or even fixation. It is economical to use for even a single specimen, and as many as 100 specimens can be handled simultaneously.†

REFERENCES

1. Baird, T. T.: A word of caution concerning dioxan. Stain Technol., **11**: 122, 1936.
2. Baird, T. T.: Comparative study on dehydration. Stain Technol., **11**: 13–23, 1936.
3. Barber, H.: Hemorrhagic nephritis and necrosis of liver from dioxan poisoning. Guy's Hosp. Rep., **84**: 267–280, 1934.
4. DeNavasques, S.: Experimental tubular necrosis of the kidneys accompanied by liver changes due to dioxan poisoning. J. Hyg., **35**: 540–548, 1935.
5. Fairley, A., Linton, E. C., and Ford-Moore, A. H.: The toxicity to animals of 1:4 dioxan. J. Hyg., **34**: 486–501, 1934.
6. Garvin, T.: The use of dioxan in histologic technic. Am. J. Clin. Path., Tech. Suppl., **2**: 175–178, 1938.
7. Graupner, V. H., and Weisberger, A.: The use of dioxan in the imbedding of microscopic objects. Zoolog. Anzeiger, **96**: 204–206, 1931.
8. Hoffmann, F. D.: Einfacher Einbettungsapparat. Zoolog. Anzeiger, **7**: 230–232, 1884.
9. Lillie, R. D.: An inexpensive apparatus for routine paraffin embedding in vacuo. Arch. Path., **16**: 232–235, 1933.
10. McClung, C. E.: Dioxan technic. Stain Technol., **11**: 121, 1936.
11. Mettler, F. A., Strong, F. C., and Mettler, C. C.: The dioxan paraffin technic. Stain Technol., **11**: 166, 1936.
12. Nelson, I. S.: The use of dioxan and acetone for the rapid dehydration and paraffin embedding of fixed tissues. Am. J. Clin. Path., Tech. Suppl., **1**: 3–7, 1937.
13. Nelson, I. A.: Wide mouth dehydrating jar with mercuric seal. Am. J. Clin. Path., Tech. Suppl., **1**: 7–10, 1937.
14. Pasternack, J. G.: A reliable one hour method for the preparation of paraffin sections of tissues. Am. J. Clin. Path., Tech. Suppl., **4**: 8–13, 1940.
15. Schrenk, H. H., and Yant, W. P.: Toxicity of dioxan. J. Indust. Hyg. and Toxicol. **18**: 448–460, 1936.
16. Vandegrift, W. B.: A dehydrating fixative for general use, including a description of technics and stains for paraffin and celloidin sections. Bull. Johns Hopkins Hosp., **71**: 96–111, 1942.
17. von Oettingen, W. F., and Jirouch, E. A.: The pharmacology of ethylene glycol and some of its derivatives in relation to their chemical constitution and physical chemical properties. J. Pharmacol. and Exper. Therap., **42**: 355–372, 1931.

* Available at the W. A. Hammond Drierite Company, Xenia, Ohio.

† This apparatus has been patented (U. S. Pat. No. 2,437,713) and may be obtained from the Will Corporation, 39 Russell Street, Rochester, N. Y.

PARAFFIN TISSUE-BLOCK TRIMMER*

MARTHA E. CONGER, M.A.

From the Genesee County Laboratory, Batavia, New York

The accompanying figure illustrates a simple device† for reducing paraffin-tissue blocks to proper size preliminary to mounting on the microtome. It consists

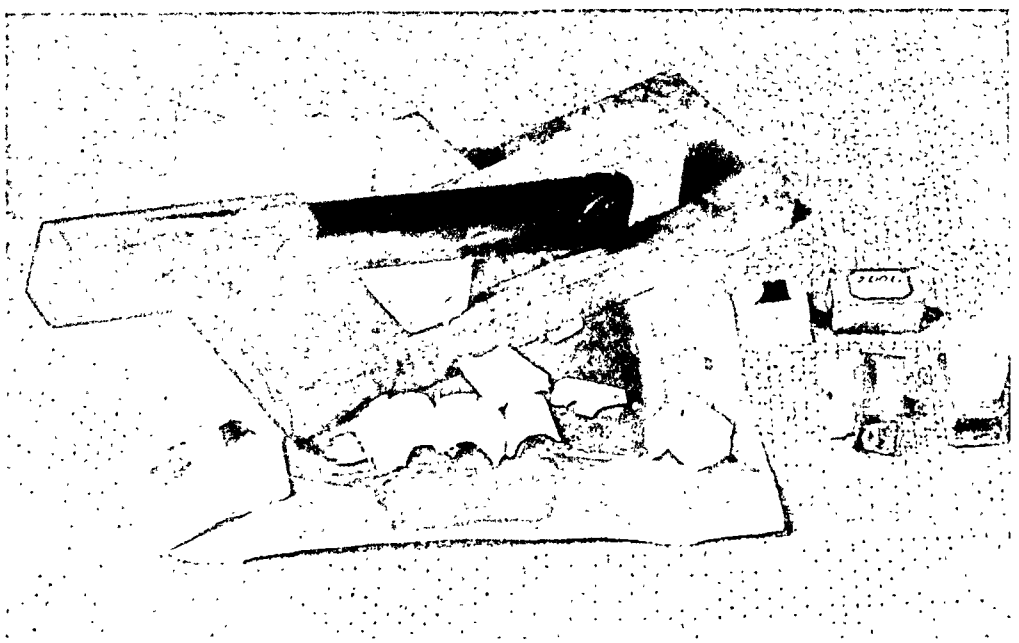


FIG. 1

of a knife blade mounted on a wooden stand, and is so pivoted that by lowering the knife, the excess paraffin can be shaved from the tissue specimen. This device has proved to be of considerable value in our laboratory after a year's trial. It saves time and eliminates fatigue of the hands of the technician who formerly had to cut the blocks with a knife.

* Received for publication, June 10, 1949.

† This apparatus may be obtained from the Will Corporation, 39 Russell Street, Rochester, New York.

CRITICAL ADSORPTION LEVELS AND ELECTROPHORETIC ANALYSES OF "PROTHROMBIN-FREE" PLASMAS*

S. GOLLUB, B.A., FRANK E. KAPLAN, B.S., DAVID R. MERANZE, M.D., AND HAROLD TUFT, M.D.

From the Laboratories of Mount Sinai Hospital of Philadelphia, Philadelphia, Pennsylvania

Plasma rendered prothrombin-free by adsorption has become widely used in blood coagulation studies. The purpose of this paper is to present data on the critical adsorption levels of three adsorbents, aluminum hydroxide [$Al(OH)_3$], barium sulfate ($BaSo_4$), and tricalcium phosphate [$Ca_3(PO_4)_2$] and an electrophoretic analysis of such adsorbed plasmas. By critical level is meant the minimum quantity of an adsorbent required to render plasma incoagulable upon addition of thromboplastin-calcium chloride with subsequent incubation at 37.5 C. for one hour.

MATERIALS AND METHODS

Oxalated normal human plasma was used throughout these experiments. Freshly precipitated and washed $BaSO_4$ and $Ca_3(PO_4)_2$ were used, since commercially available reagents were inconsistent in activity and required more material for adsorption. Tricalcium phosphate was prepared by mixing calcium chloride ($CaCl_2$) slowly with trisodium phosphate (Na_3PO_4) at a cool temperature, stirring constantly. Then the precipitate was washed with distilled water until the supernate failed to show a positive test for the chloride ion (Cl^-) with silver nitrate in the presence of nitric acid. It was finally made up as a stock, calculated 10 per cent suspension with distilled water. Barium sulfate was similarly prepared by mixing calcium chloride with sulfuric acid and made up as a stock, calculated 20 per cent suspension with distilled water. Aluminum hydroxide cream was used in the colloidal form† as a stock 5 per cent suspension. These stock suspensions were subsequently diluted with distilled water to various concentrations. Table 1 lists those concentrations found to be closest to the critical level when added in 2 ml. amounts to 10 ml. of oxalated plasma.

The plasma-adsorbent mixture was incubated at 37.5 C. in a water bath for ten minutes. While in the water bath, the mixture was shaken once each minute to redisperse the adsorbent. Following hard centrifugation, the clear plasma was taken off. One-tenth ml. of this plasma was mixed with 0.2 ml. of a 1:1 mixture of thromboplastin-calcium chloride. The mixture was incubated at 37.5 C. in a water bath for one hour. At the end of this period the tube was examined for a clot.

The adsorbents were assayed on each of three normal human plasmas. Electrophoretic analyses were made on two of these plasmas.

* Received for publication, August 13, 1949.

† Amphojel, a commercial preparation of 5 per cent aluminum hydroxide. Product of Wyeth, Inc., Philadelphia 3, Pa.

TABLE I

Adsorption Levels for Normal Human Plasmas

Adsorption of Prothrombin from 10 ml. of Each of Three Oxalated Plasmas by 2 ml. of indicated Percentages of Suspensions of Adsorbents

Plasma 1			Plasma 2			Plasma 3		
$Al(OH)_3$	$BaSO_4$	$Ca_3(PO_4)_2$	$Al(OH)_3$	$BaSO_4$	$Ca_3(PO_4)_2$	$Al(OH)_3$	$BaSO_4$	$Ca_3(PO_4)_2$
1%	10%	0.5%	1%	10%	1%	1%	10%	1%
±*	−	−	−	−	−	−	−	−
0.75%	5%	0.25%	0.75%	7.5%	0.75%	0.75%	7.5%	0.75%
+	+	+	+	+	+	−	+	−
0.5%	2%		0.5%	5%	0.50%	0.5%		0.5%
+	+		+	+	+	+		+

* ± indicates slight web-like clot observed after one hour's incubation; +, clot observed after one hour's incubation; −, no clot observed.

TABLE 2

Comparison of Adsorbed and Unadsorbed Plasma Schlieren Diagrams

(Figures in table refer to percentage areas)

Plasma*	Albumin	Alpha 1 Globulin	Alpha 2 Globulin	Beta Globulin	Fibrinogen	Gamma Globulin
			Unabsorbed			
#1—a	53.5	4.71	10.1	10.8	9.06	11.7
—b	56.3	6.11	7.03	9.1	9.35	12.1
#2—a	62.8	4.41	8.00	10.2	6.21	8.4
—b	63.3	3.48	8.17	10.4	5.23	9.4
—c	62.0	4.10	8.90	11.0	6.10	7.8
—d	57.4	4.56	11.30	7.78	9.52	11.4
			Averages			
	58.9	5.63	8.91	8.92	7.58	10.1
			Absorbed			
#1—e	56.8	4.6	6.4	11.4	9.6	11.3
—f	49.5	4.7	7.1	12.3	12.2	12.7
—g	55.8	3.4	6.7	12.8	9.5	14.4
#2—e	56.9	4.4	10.8	10.7	6.9	10.3
—f	55.5	6.2	9.4	10.6	7.5	10.9
—g	59.5	4.9	8.7	12.2	6.1	8.1
—h	66.4	3.5	6.9	10.0	6.2	7.0
—j	55.5	4.8	9.8	11.3	8.8	10.1
			Averages			
	56.3	4.75	8.95	11.4	8.35	10.6

* Plasmas a, b, c and d were unadsorbed plasmas; e, plasmas adsorbed with Amphojel; f, plasmas adsorbed with $BaSO_4$; and g, h and j, plasmas adsorbed with $Ca_3(PO_4)_2$.

For electrophoretic analysis,[1, 2, 5, 8] 5 ml. of the plasmas listed in Table 2 were diluted with 10 ml. of buffer and dialyzed against the buffer for 24 hours. A Klett electrophoresis apparatus was employed using the Schlieren scanning method of recording. An 11-ml. one-piece center-section cell was used employing a sodium veronal buffer of pH 8.6, ionic strength (μ) = 0.1 in a water bath kept constant at 1 C. Areas were measured by planimetry of an eleven-fold (area) increased pattern. The figures in Table 2 refer to the ascending patterns.

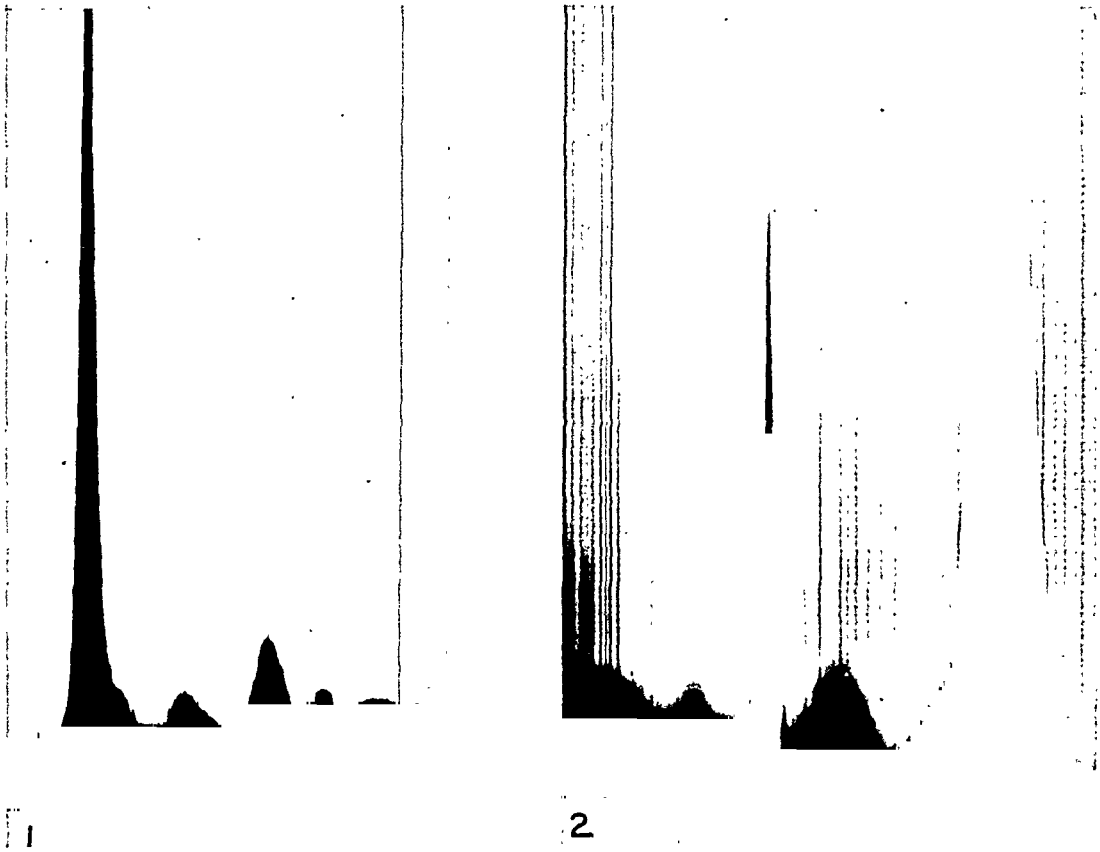


FIG. 1. Sample pattern of ascending boundary
FIG. 2. Sample pattern of descending boundary

RESULTS

Table 1 shows adsorption levels for the three different plasmas. It is seen that the variation of critical adsorption values among the different plasmas is small and that the highest critical adsorption value found for each adsorbent was 1 per cent for $Al(OH)_3$, 10 per cent for $BaSO_4$ and 1 per cent for $Ca_3(PO_4)_2$.

Since 2 ml. of each suspension was used for 10 ml. plasma, the critical level on a dry-weight basis of the above plasmas was calculated to be approximately as follows: $BaSO_4$, 2.0 Gm./100 ml. plasma; $Ca_3(PO_4)_2$, 0.2 Gm./100 ml. plasma; $Al(OH)_3$, 0.2 Gm./100 ml. plasma.

Results of electrophoretic analyses are shown in Table 2. Neither the absolute amounts of the plasma proteins nor the relative percentage of the adsorbed plasma was found to be significantly different from unadsorbed plasma by simple statistical analysis (Student's "T" distribution). Samples of the electrophoretic pattern are included (Figs. 1 and 2).

DISCUSSION

The critical adsorption values reported here for $BaSO_4$ and $Al(OH)_3$ were similar to those reported by Fantl and Nance.[6] We have found in supplementary tests with several plasmas that their critical adsorption values fluctuated about the values reported here. As was expected, the electrophoretic technic failed to detect the nature and amount of the plasma fraction, the removal of which by adsorption rendered the plasma incoagulable. From the data presented, it is seen that the gross electrophoretic patterns were not altered by critical adsorption. This seems to indicate that adsorbed plasma may be used as a whole plasma diluent in studies involving prothrombin and other coagulation components without altering the gross plasma protein system. Almost all the earlier workers[3, 4, 8, 10, 11] used large excesses of adsorbent. Our results show that such high concentrations are unnecessary. Indeed, large excesses of adsorbent significantly alter the Schlieren patterns of plasma,[12] so that it seems advisable to keep the amount of adsorbent close to the critical level in order to retain the essential characteristics of whole plasma.

SUMMARY

Critical adsorption levels for three prothrombin adsorbents are presented together with the electrophoretic analyses of the plasmas so adsorbed. These data show: (1) The quantitative adsorptive power of barium sulfate, calcium phosphate and aluminum hydroxide on three different plasmas; (2) that different adsorbents have different critical levels; (3) that at the critical adsorption level, the plasma electrophoresis patterns are the same regardless of the adsorbent employed; (4) that the electrophoretic integrity of plasma is not impaired by critical adsorption.

Acknowledgments. We are indebted to Dr. Ralph Siu and Dr. William H. Stahl of the Philadelphia Quartermaster Depot for the use of the electrophoretic equipment employed in this work.

REFERENCES

1. Abramson, H. A., Moyer, L. S., and Gorin, M. H.: Electrophoresis of Proteins and the Chemistry of Cell Surfaces. New York: Reinhold Publishing Co., 1942.
2. Alberty, R. A.: An introduction to electrophoresis. J. Chem. Ed., **25**: 426–433, 619–625, 1948.
3. Bordet, J., and Delange, L.: La coagulation du sang et la genese de la thrombine. Ann. Inst. Pasteur, **26**: 657–674, 737–766, 1912.
4. Dale, H. H., and Walpole, G.: Some experiments on factors concerned in the formation of thrombin. Biochem. J., **10**: 331–362, 1916.
5. Electrophoresis (Symposium). Ann. N. Y. Acad. Sc., **39**, 105–212, 1939.

6. Fantl, P., and Nance, M. H.: The relation between plasma coagulation time and prothrombin concentration. Australian J. Exper. Biol. and M. Sc., **25**: 95–101, 1947.
7. Fuchs, H. J.: Wichtige methodische Einzelheiten bie Blutgerinnungsuntersuchungen sowie eine Isolierungsmethodik des physiologischen gerinnungshemmenden Faktors aus Blut und Gewebe. Biochem. Ztschr, **222**: 470–481. 1930.
8. Quick, A. J.: The Hemorrhagic Diseases and the Physiology of Hemostasis. Springfield, Ill: Charles C Thomas, 1942.
9. Rosenfield, R. E., and Tuft, H. S.: Estimation of prothrombin level from prothrombin time. Am. J. Clin. Path., **17**: 405–412, 1947.
10. Stern, K. G., and Reiner, M.: Electrophoresis in medicine. Yale J. Biol. and Med., **19**: 67–99, 1946.
11. Tanturi, C. A., and Banfi, R. F.: An improved method for the determination of plasma prothrombin. J. Lab. and Clin. Med., **31**: 703–713, 1946.
12. Unpublished data (Laboratories of Mount Sinai Hospital of Philadelphia).

AN IMPROVED APPARATUS FOR THE MICRODETERMINATION OF CHOLESTEROL*

E. M. ABRAHAMSON, M.D.

From the Jewish Hospital, Brooklyn, New York

Cholesterol is usually determined colorimetrically, using the Liebermann-Burchard reaction[6] to develop the color. The procedures in common use differ only in methods of obtaining a solution of cholesterol in chloroform which is treated with acetic anhydride and sulfuric acid to produce a green color.

When other lipoid constituents of the blood are to be determined, Bloor's method,[3] in which the sample is treated with a mixture of alcohol and ether, is generally used. The non-lipoid material is precipitated and an aliquot part of the filtered solution is used to determine the cholesterol. The assumption that the cholesterol is uniformly dispersed between the solid and liquid phases of the mixture is unsound theoretically; also there is the practical objection that it is impossible to filter a liquid as volatile as ether without obtaining significant concentration of the solution by evaporation.

Since the cholesterol is the only lipoid of routine clinical importance, direct extraction methods which avoid the inaccuracies mentioned above are preferable. The method of Myers and Wardell[8] has become standard. The sample is mixed with Plaster of Paris in a suitable vessel and the mixture is then dried in the oven to remove the water. The dry mixture is transferred to an extraction thimble and extracted with chloroform in a modified Soxhlet apparatus. The extract is made up to a fixed volume with chloroform, and an aliquot part is treated in a test tube with acetic anhydride and sulfuric acid in the usual manner.

Because of the large sample required (1.0 ml.) and the difficulty in transferring the blood-plaster mixture quantitatively, several modifications of this method have been proposed. Leiboff[5] simplifies the method by absorbing the sample on a disk of filter paper, drying it with gentle heat and extracting the cholesterol in a special apparatus in which the color development later takes place without removal of the extract. This method avoids error due to evaporation of the solvent during transfer. Ling[7] has a similar apparatus. The author[1] devised an apparatus in which the blood was absorbed on cotton, extracted and treated to develop the color in the same apparatus.

The problem of drying the sample becomes very important in these four methods. If drying is incomplete, the solvent (chloroform) is kept from intimate contact with all parts of the sample by surface tension and extraction may be incomplete. If the sample is dried too rapidly, it tends to form a varnish-like glaze on its surface which may entrap some water and also act as an impervious skin to keep the solvent away from the innermost portions of the sample.

All of these direct extraction methods were tried in duplicate, using dried and

* Received for publication, August 19, 1949.

undried samples, and the results were frequently discordant. There was no regularity in the discrepancies. In some cases the dried sample gave the higher value, showing that extraction was more complete; in as many others, the opposite result was obtained.

The method in which the samples were taken up on filter paper or cotton showed some solutions which were "not true" in color. This tended to occur in samples containing bile pigments. Myers's claim that calcium sulfate retains interfering substances is probably correct.

In order to avoid all these difficulties, the author has modified his apparatus. In its present form it is not necessary to dry the sample and the resultant color is identical in shade to that of the standard. It has the additional advantage of requiring absolutely no handling of the sample once it is introduced into the apparatus.

APPARATUS*

An extraction tube: a glass-stoppered test tube 200 x 20 mm. with a constriction 50 mm. from the bottom

A micro-extractor which rests on the constriction

A condenser ground to fit the mouth of the extraction tube and provided with a longitudinal groove in the ground joint which serves as a vent to prevent the condenser from being forced out of the extraction tube

A drying tube, also ground to fit the extraction tube and fitted with a calcium chloride tube

A suitable source of heat

Plaster cylinders to absorb and dry the sample

5, 2 and 0.2 ml. pipets, the latter of the Mohr variety

Glass beads

The plaster cylinders are prepared from a thick paste of Plaster of Paris and water by thrusting one end of a piece of 5 mm. (i.d.) glass tubing into the paste and connecting the other end to a vacuum pump by means of heavy rubber tubing (Fig. 1). The paste is drawn up, without any air bubbles, into the glass tube to form a cast of the tube. The filled tube is disconnected from the pump and allowed to stand on its side overnight for the plaster to set. It is then broken into pieces of convenient length which are placed in a furnace heated to 500 C. for a working day, to dehydrate the calcium sulfate completely. The glass tubes with their plaster contents are allowed to cool in a desiccator. Then, the plaster cylinders are removed by pushing them out of their sheaths with a metal rod. They slip out of the glass easily. Finally, the cylinders are broken into pieces about 2 cm. in length and stored in a tightly-stoppered bottle.

REAGENTS

Absolute alcohol

Anhydrous chloroform

* The glass apparatus was blown from Pyrex glass by Eimer and Amend of New York City.

Acetic anhydride
Concentrated sulfuric acid
Standard cholesterol solution 0.08 mg. cholesterol per ml. in chloroform. This solution must be kept in a tightly-stoppered bottle to prevent evaporation of the solvent. For the same reason it should be prepared fresh frequently.

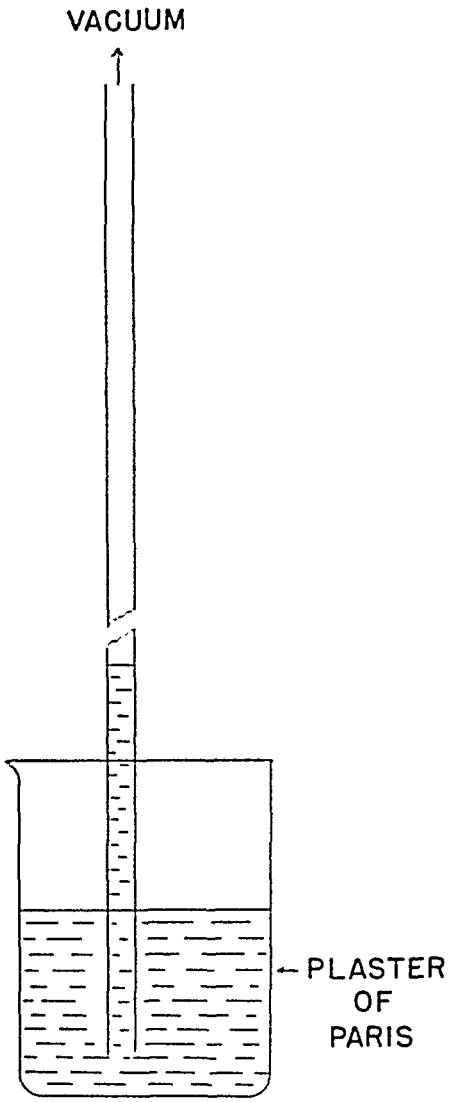


FIG. 1. Preparation of plaster cylinders

PROCEDURE

1. A cylinder of plaster is placed in a micro-extractor.
2. 0.2 ml. of serum is taken up in a pipet, the tip of which is placed against the end of the cylinder. The other end of the pipet is raised gradually without disturbing the contact between the tip and the plaster cylinder. The serum is absorbed quantitatively by the plaster.
3. A glass bead is dropped into the extraction tube and 5 ml. of absolute alcohol is added.
4. The extractor is lowered into position by means of a hook, made from a piece of stiff wire, to engage the syphon of the extractor.
5. The condenser is placed in the mouth of the extraction tube and connected with the water supply.
6. The apparatus is placed on the heater, a suitable form being a cigar-lighter heating element connected to the 110-volt mains in series with a 200-watt Mazda lamp whose resistance lowers the current so that boiling is smooth and the life of the element prolonged. The lamp also serves as an indicator to show that the heat is turned on. A brass cylinder, 5 cm. long, cut from $1\frac{3}{8}$ inch brass tubing rests on the porcelain receptacle. It conserves heat and protects the extraction tube from draughts. Some of the heating elements on the market have automatic switches so that they will heat only when tipped from the vertical position. Such elements will work in any position if a nail is cut off 2 cm. from its head and inserted into the hole at the bottom of the element. A more elegant heater is the "Hot Spotter" made by the Fisher Scientific Company. A gas micro-burner is also satisfactory. The apparatus is thus assembled as shown in Figure 2. The ground joints are indicated by the heavy lines. The extraction is

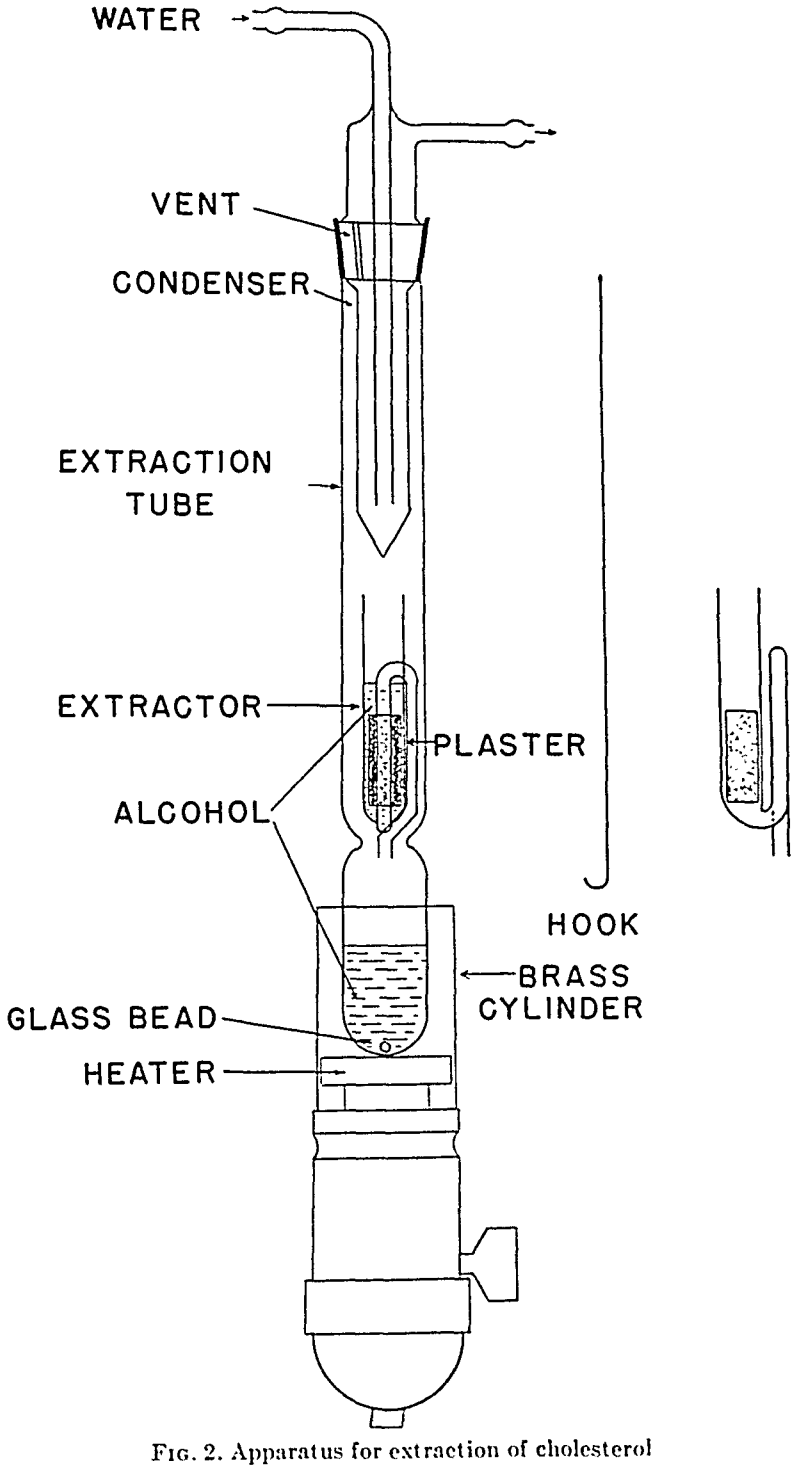


FIG. 2. Apparatus for extraction of cholesterol

allowed to proceed for a minimum of two hours which is sufficient to extract all the cholesterol. The apparatus requires no attention except for a glance now and then to see that the water is flowing in the condenser.

7. The condenser is removed from the extraction tube. Then the extractor is removed by lifting it out with the wire hook. The drying tube is inserted as

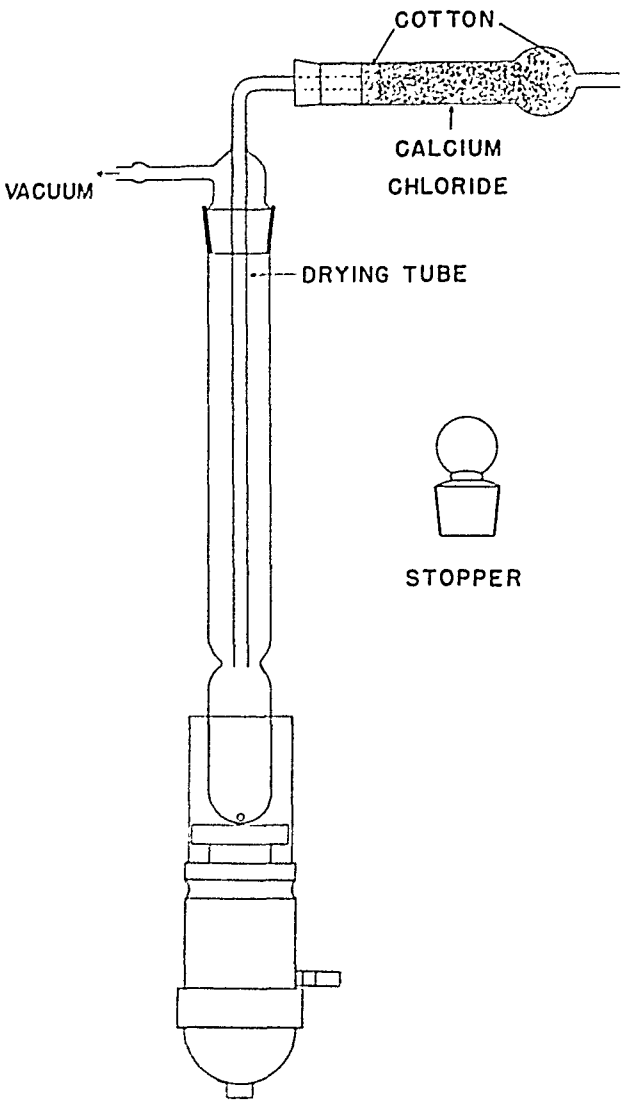


FIG. 3. Evaporation of extraction menstruum

shown in Figure 3. A current of dried air is drawn through the apparatus until almost all of the alcohol is evaporated. Then the electric current is turned off and the residual heat in the element and brass cylinder will complete evaporation without danger of charring the extract.

8. The apparatus is dismantled and the extraction tube is stoppered and set aside to cool. The glass bead is allowed to remain. The cholesterol can be seen coating the wall of the lower chamber of the extraction tube.

9. When the tube is cool, 5 ml. of chloroform and 2 ml. of acetic anhydride are added and the stoppered tube is swirled to dissolve the cholesterol; 0.1 ml. of concentrated sulfuric acid is added.

10. A standard is prepared by placing 5 ml. of standard cholesterol solution, 2 ml. of acetic anhydride and 0.1 ml. of concentrated sulfuric acid in a glass stoppered test tube. The standard and unknown are allowed to stand in a dark closet for 15 minutes and then compared.

Calculation. Mg. cholesterol per 100 ml. of serum $= \frac{S}{U} \times 200.$

DISCUSSION

The cylinder of anhydrous calcium sulfate is a good dehydrating agent and the water in the serum is readily absorbed. By using absolute alcohol as the extraction menstruum, we insure complete extraction. Five ml. of absolute alcohol can absorb 0.25 ml. of water to become the ordinary 95 per cent alcohol. This amount is greater than the total volume of the sample. The drying of the sample, therefore, is no problem. Although the cylinder will absorb whole blood, it does so more slowly than it absorbs serum and it is easy to secure enough blood by finger puncture to obtain 0.2 ml. of serum. If small tubes are made from glass tubing, 0.5 ml. of blood can be centrifuged to yield enough serum for this determination. Even with icteric bloods the calcium sulfate will fix the bile pigments so that the green color can be matched with that of the standard.

With the proportions of the reagents chosen, it is possible to use Myers's β-naphthol green standard. An 0.005 per cent aqueous solution set at 15.5 mm. in an optical colorimeter is equivalent to the standard cholesterol set at 15 mm. The Klett glass standard can also be used. Both of these are for the optical colorimeter. The calculations are the same.

Once the colorimeter has been standardized, it is possible to reduce the size of the sample still further, to 0.1 ml. If this is found to be desirable, the procedure is modified slightly.

After extraction and evaporation of the alcohol are completed, 5 ml. of a mixture, freshly made from 5 ml. of chloroform and 2 ml. of acetic anhydride, is added to the extraction tube. Then 0.07 ml. of concentrated sulfuric acid is added and the color is read after standing in the dark for 15 minutes. The calculation then becomes:

$$\text{Mg. cholesterol per 100 ml. of serum} = \frac{S}{U} \times \frac{2000}{7}$$

This calculation can be made because, when 5 ml. of chloroform is added to 2 ml. of acetic anhydride, the total volume is 7 ml. without appreciable shrinkage. This modification was tried in duplicate with the double sample and the difference was insignificant.

This modification is particularly useful with the photoelectric colorimeter or with the artificial standards in the optical colorimeter. It reduces the number of volumetric measurements, thus removing one chance for error.

This apparatus was used in over 200 duplicate analyses with and without

added cholesterol and the agreement in all cases was within 3 per cent which is well within the error of the colorimeter and is certainly satisfactory for clinical purposes.

Acknowledgment. I wish to acknowledge the assistance of my technician, Yolanda Sasso, who performed many of the analyses upon which this paper is based.

REFERENCES

1. Abrahamson, E. M.: An apparatus for the microdetermination of cholesterol in blood. Science, **86**: 477–478, 1937.
2. Abrahamson, E. M.: The use of cotton to absorb blood for cholesterol extraction. Science, **87**: 420, 1938.
3. Bloor, W. R.: The determination of cholesterol in blood. S. Biol. Chem., **24**: 227–231, 1916.
4. Burchard, H.: Beitrage zur Kenntnis der Chlosterine. Dissertation, Rostock, 1889.
5. Leiboff, S. L.: A simplified method for cholesterol determination in blood. J. Biol. Chem., **61**: 177–180, 1924.
6. Liebermann, C.: Über das Oxychinoterpin. Ber. d. deutsch. chem. Gesellsch., **18**: 1803–1810, 1885.
7. Ling, S. M.: The determination of cholesterol in small amounts of blood. J. Biol. Chem., **76**: 361–365, 1928.
8. Myers, V. C., and Wardell, E. L.: The colorimetric estimation of cholesterol in blood. J. Biol. Chem., **36**: 147–156, 1918.

PENICILLIN-RESISTANT STAPHYLOCOCCI*

W. G. RICE, M.D.,† AND A. MERCEDES LONERGAN, R.T. (CANADA)

From the Laboratories of King's Daughters Clinic and Hospital, Temple, Texas

The incidence of bacteria that are resistant to antibiotics is increasing. This is particularly true of the resistance of the pathogenic staphylococcus to penicillin.[1, 8] Some naturally occurring strains of staphylococci are resistant to penicillin,[2] or may give rise to resistant mutants when exposed to sublethal concentrations of penicillin.[3, 5, 7] Barber[1] has reported an increase from 14.1 to 59 per cent of resistant strains of staphylococci encountered in a hospital laboratory. Theoretically, in a community when penicillin is frequently used, there should soon be a measurable increase in the number of resistant bacteria. The practical importance of this increase is obvious.

Most of the investigations of resistant staphylococci have been made on selected material submitted for laboratory diagnosis. However, in 1949, Martin and Whitehead[6] reported a survey of pathogenic staphylococci obtained from 50 medical students in a London hospital. For purposes of comparison, the present survey was conducted on a similar basis. There are, however, two differences: the geographic location, and the increased proportion of persons who had previously received penicillin.

PROCEDURE

Specimens were secured from 50 unselected volunteers from the staff of King's Daughters Hospital. Thirty-four of these persons were hospital attendants, nurses, and nursing aides, who were in close association with patients. They were classified as "contacts". The remaining 16 had no direct contact with patients. Swabs were taken for culture from each person from three sites: (1) throat, a single dry swab; (2) nostrils, a single, broth-moistened swab from both nostrils; and (3) skin of face, a single broth-moistened swab, rotated approximately 20 times over both malar prominences, over an area 3 cm. in diameter.

Swabs were incubated in beef infusion broth for 18 to 24 hours. The broth was subcultured on human blood agar plates, incubated for 24 hours and examined for colonies of staphylococci. Colonies were selected on the basis of visible differences of form, pigment production and hemolysis, and subcultured on segments of blood agar plate for 12 to 18 hours. Single colonies were picked and examined microscopically. Colonies of staphylococci were then subcultured to plain agar stabs, incubated 12 to 18 hours and stored at 2 to 4 C for from 9 to 22 days. The blood agar plates were left at room temperature for three days and examined daily for pigment formation. One hundred and fifteen colonies were collected on stabs. All of these were tested for coagulase (Gillespie's method),[4] and at the same time tested for resistance to penicillin[2] and streptomycin.

Blood agar plates were prepared with parallel gutters 2–3 mm. deep and 7–9 mm. wide. The gutters were filled with blood agar containing fresh solutions of commercial penicillin (Buffered Penicillin-G Sodium, Lederle) and streptomycin (Streptomycin Merck, Calcium Chloride Complex). Penicillin in a concentration of 10 units per ml. inhibited a standard

* Received for publication, June 3, 1949.

† Present address, William Buchanan Blood Center, Dallas, Texas.

TABLE 1

DISTRIBUTION AND CHARACTERISTICS OF STAPHYLOCOCCI (*Micrococcus pyogenes*) IN 50 NORMAL PERSONS

SITE CULTURED	COLONIES OF STAPHYLOCOCCI STUDIED	COAGULASE-POSITIVE AUREUS COLONIES	ALL TYPES PENICILLIN-RESISTANT COLONIES	PENICILLIN-RESISTANT PATHOGENS
Nostrils	37	20	21	8
Skin of face	58	18	17	13
Throat	20	16	8	9
Total	115	54	46	30

TABLE 2

COMPARISON OF RESISTANCE TO PENICILLIN OF STAPHYLOCOCCI ISOLATED WITH STANDARD ORGANISM (*M. pyogenes* VAR. *aureus* #9996 SM STRAIN)

CASE NO.	COLONY*	INHIBITED BY U/ml.	× STANDARD
Standard		0.007	1
1	A	250	35000
	B	500	70000
	C	125	17500
2	B	62.5	8750
	C	62.5	8750
3	A	125	17500
5	B	500	70000
11	A	62.5	8750
	B	250	35000
13	B	500	70000
14	B	250	35000
	C	250	35000
15	A	500	70000
	B	500	70000
	C	15.6	2200
19	C	125	17500
29	A	250	35000
	B	250	35000
31	A	31.25	4400
34	A	15.6	2200
	B	62.5	8750
	C_1	62.5	8750
	C_2	62.5	8750
39	A	62.5	8750
	B	125	17500
	C	500	70000
43	B_1	125	17500
	B_2	500	70000
48	B	125	17500
	C	250	35000

* A, culture from throat; B, from nose; C, from face.

staphylococcus for 14–18 mm. in six different plates (*Micrococcus pyogenes* var. *aureus* 9996, W. A. Randall FDA Strain SM, from the American Type Culture Collection). Streptomycin, in a concentration of 500 units per ml., inhibited the same organism from 6 to 11 mm. in different plates. The unknown strains of staphylococci were streaked on these test plates and incubated for 24 hours. Resistance was assigned to a strain when the organism grew directly across the gutter. In a number of instances organisms grew within the zone of control inhibition. These were not included with the resistant strains, since some susceptibility was exhibited.

Thirty penicillin-resistant strains were recovered. These were tested by culture in serial dilutions of penicillin in broth. The inoculum in each instance was 0.02 ml. of a 24-hour broth culture. The resistance was then compared with the resistance of the standard strain of staphylococcus under identical conditions.

RESULTS

One hundred and fifteen colonies of staphylococci were isolated from the 50 persons investigated. Fifty-four (47 per cent) of these were coagulase-positive pigment producers and considered to be potentially pathogenic. Thirty (55.5 per cent) of the 54 were completely resistant to penicillin, which is 26 per cent of all staphylococci isolated. The figures showing the general distribution of colonies are listed in Table 1. No attempt was made to identify the colonies further so that it is not possible to state categorically that in some instances they are not mutants of the same strain. This is particularly true when more than one colony was obtained from a swab or even when more than one colony was obtained from a single individual.

Sixteen penicillin-resistant colonies were coagulase-negative and considered to be non-pathogenic. Fifteen of these 16 colonies were *M. pyogenes* var. *albus*, and one was *M. pyogenes* var. *aureus*. The 54 strains of pathogenic staphylococci were carried at one or more of the three sites by 26 persons (52 per cent). The 30 colonies of penicillin-resistant bacteria were carried by 15 persons (30 per cent of those tested). Both susceptible and resistant colonies were found in two instances. There was a fairly uniform distribution of resistant and nonresistant colonies and no predilection for any one of the sites was found. Penicillin-resistant staphylococci were carried by 2 of the 16 "noncontacts" (12.5 per cent) and by 13 of the 34 "contacts" (37 per cent). This is a striking difference.

Thirty-four persons had had previous penicillin medication. These were divided evenly between the 26 carriers and the 24 noncarriers. Seven of 16 "noncontacts" (44 per cent) were carriers of pathogenic staphylococci while 19 of 34 "contacts" (56 per cent) were carriers. This is not a significant difference.

The relative resistance of the 30 colonies was tested by serial dilution method and compared with a standard strain (*M. pyogenes* var. *aureus* #9996 SM strain). Growth of the standard staphylococcus was inhibited by 0.007 units per ml. of penicillin (Table 2). Growth of resistant colonies occurred in a concentration as high as 500 units per ml. Streptomycin-resistance was exhibited by only one of the 115 colonies. This was a coagulase-negative nonpigment-producing organism classified as *M. pyogenes* var. *albus*, recovered from the face of a "contact" who had previously had both penicillin and streptomycin. The organism was also resistant to penicillin. The 54 colonies of pathogenic staphylococci

showed from 5 to 10 mm. of inhibition to streptomycin; with the exception of one penicillin-resistant colony which was only inhibited for 2 mm.

TABLE 3

SHOWING NUMBER OF MICROGRAMS OF AUREOMYCIN HYDROCHLORIDE PER ML. OF AGAR REQUIRED TO INHIBIT GROWTH OF VARIOUS STAPHYLOCOCCI

STAPHYLOCOCCUS COLONY NO.*	NUMBER OF MICROGRAMS PER ML. AGAR**						
	2.5	1.25	0.6	0.3	0.15	0.07	0.035
1A	−	−	−	−	−	+	+
1B	−	−	−	+	+	+	+
1C	−	−	−	+	+	+	+
2B	−	−	−	+	+	+	+
2C	−	−	−	+	+	+	+
3A	−	−	−	+	+	+	+
5B	−	−	+	+	+	+	+
11A	−	−	−	+	+	+	+
11B	−	−	−	+	+	+	+
13B	−	−	−	+	+	+	+
14B	−	−	−	−	+	+	+
14C	−	−	−	+	+	+	+
15A	−	−	−	−	+	+	+
15B	−	−	−	+	+	+	+
15C	−	−	−	−	+	+	+
19C	−	−	−	−	+	+	+
29A	−	−	+	+	+	+	+
29B	−	−	−	−	+	+	+
31A	−	−	−	+	+	+	+
34A	−	−	−	−	−	+	+
34B	−	−	−	−	+	+	+
$34C_1$	−	−	−	−	+	+	+
$34C_2$	−	−	−	−	+	+	+
39A	−	−	−	−	+	+	+
39B	−	−	−	−	+	+	+
39C	−	−	−	−	+	+	+
$43B_1$	−	−	−	−	+	+	+
$43B_2$	−	−	−	−	+	+	+
48B	−	−	−	−	+	+	+
48C	−	−	−	−	+	+	+
Standard	−	−	−	−	+	+	+
Standard	−	−	−	−	+	+	+

* Culture from throat; B, from nose; C, from face.

** − No growth; + growth.

DISCUSSION

Comparison was made with the results obtained by Martin and Whitehead,[6] limiting their figures to colonies recovered from nostrils, face and throat. From these sites, they found 43 colonies of *Staph. pyogenes*, 11 of which (25.8 per cent) were penicillin-resistant and were carried by 5 persons (10 per cent of the entire group). In the present series 54 colonies of pathogenic staphylococci were found.

This is not a significant difference. However, 30 of these (55.5 per cent) were resistant to penicillin; they were carried by 15 persons (30 per cent of the entire group). There were, therefore, more than twice as many resistant colonies and three times as many carriers.

This difference may be due to geographic variation in strains of staphylococci or the general use of penicillin. Sixty-eight per cent of all subjects tested had had penicillin medication, in many instances in low concentrations or for a short time. However, carrying of resistant bacteria by the individual was apparently not related to previous penicillin treatment. Experiments have shown that resistant mutants are likely to survive if the early penicillin concentrations are not high.[2] There is a strong probability that this factor has influenced the high general incidence of penicillin-resistant bacteria. The high carrier rate among hospital attendants in contact with many persons receiving penicillin may be of some significance.

In the small group surveyed these figures have little statistical significance, but may possibly serve to indicate that the number of penicillin-resistant strains of bacteria is increasing. In successful treatment the proper choice of an antibiotic becomes of increasing importance. It is not only necessary for the laboratory to identify the pathogen but frequently it is also necessary to give an estimate of its susceptibility to antibiotics.

SUMMARY

1. In a survey of 50 persons, from whom cultures were made from the nostrils, throat and skin, 26 were found to be carriers of potentially pathogenic staphylococci (*Micrococcus pyogenes* var. *aureus*).

2. Fifteen persons carried *M. pyogenes* var. *aureus* which was completely resistant to penicillin.

3. There was no striking difference in percentage of persons from whom staphylococci were recovered among those who were and those who were not in contact with patients. Neither was there a striking difference in recovery of these organisms from among those who had received penicillin previously and from those who had not received penicillin. Thirty-four persons had previously had penicillin.

4. Thirteen (37 per cent) "contacts" carried penicillin-resistant colonies while only two (12.5 per cent) "noncontacts" carried similar colonies.

5. Comparison with a previous similar English report shows more than double the percentage of penicillin-resistant colonies and triple the percentage of carriers.

6. Resistant colonies were not inhibited in serially-diluted broth cultures by concentrations of penicillin up to 500 units per ml.

7. Of 115 colonies of staphylococci only one, a nonpathogen, exhibited complete resistance to streptomycin.

REFERENCES

1. Annotations: Resistant staphylococci. Brit. M. J.: 64, 1949.
2. Barber, M.: Coagulase-positive staphylococci resistant to penicillin. J. Path. and Bact., 59: 373–384, 1947.
3. Demerec, M.: Origin of bacterial resistance to antibiotics. J. Bact., 56: 63–74, 1948.

4. KNOTT, F. A.: Laboratory control of pathogenic staphylococci. In: Recent Advances in Clinical Pathology. S. C. Dyke, Editor. Philadelphia: The Blakiston Co., pp. 89–98, 1947.
5. LANKFORD, C. E., AND LACY, H.: In vitro response of staphylococcus to aureomycin, streptomycin and penicillin. Texas Rep. Biol. and Med., 7: 111–124, 1949.
6. MARTIN, T. D. M., AND WHITEHEAD, J. E. M.: Carriage of penicillin-resistant *Staph. pyogenes* in healthy adults. Brit. M. J.: 173–174, 1949.
7. PARKER, R. F., AND LUSE, S.: The action of penicillin on staphylococcus; further observations on the effect of a short exposure. J. Bact., 56: 75–81, 1948.
8. SPINK, W. W., HALL, W. H., AND FERRIS, V.: Clinical significance of staphylococci; with natural or acquired resistance to sulfonamides and to penicillin. J. A. M. A., 128: 555–559, 1945.

SUPPLEMENTAL REPORT ON RESISTANCE TO AUREOMYCIN

Standard commercial preparations of aureomycin were not suitable for testing bacterial resistance. Soluble aureomycin hydrochloride* was used to test the sensitivity of the 30 strains of penicillin-resistant staphylococci.

Technic. Serial dilutions of aureomycin hydrochloride in glucose Beef Infusion broth were made, with a concentration range from 5 to 0.035 micrograms per ml. Each tube was inoculated with approximately 0.02 ml. of a 24-hour broth culture of the organisms, and incubated for 18 hours. The results are listed in Table 3. In addition a number of the organisms were tested for inhibition by aureomycin on a ditch plate. The agar in the ditch contained 10 micrograms per ml. of aureomycin hydrochloride.

Results. No significant resistance to aureomycin was encountered in any of the 30 penicillin-resistant colonies tested. The majority were inhibited by concentrations of 0.3 to 0.6 micrograms (maximum of 1.25 and minimum of 0.15). A standard strain of Staphylococcus (*Micrococcus pyogenes* var. *aureus* SM strain from American Type Culture Collection) was inhibited consistently by 0.3 micrograms per ml. Ten of the colonies of staphylococci and one standard strain tested on agar ditch plates showed zones of inhibition varying from 7 to 9 mm. in diameter. These same colonies were inhibited in broth by concentrations of the antibiotic ranging from 0.07 to 0.6 micrograms per ml. of broth.

REFERENCE

1. DORNBUSH, A. C.: Aureomycin Laboratory Methods. Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

* Supplied by Dr. Benjamin W. Carey, Lederle Laboratories Division, American Cyanamid Company.

AN IMPROVISED MICROTOME KNIFE SHARPENER*

W. G. RICE, M.D.

From the William Buchanan Blood Center, Dallas, Texas

A simple mechanical microtome knife sharpener may be constructed using an LP (long-playing) record-player turntable and a circular piece of plate glass.

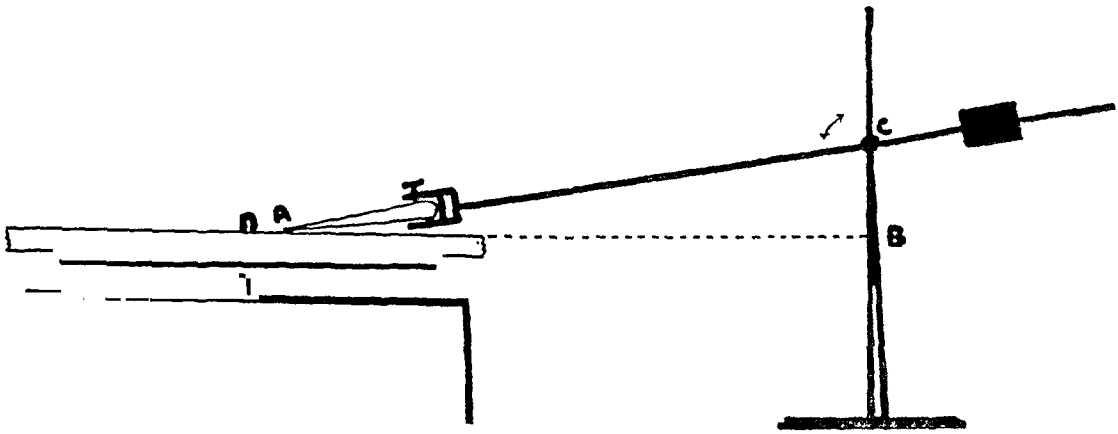


FIG. 1. Diagram of arrangement of apparatus using LP record turntable. Note triangle ABC from which angle of bevel may be adjusted and calculated.

The turntable should be mounted on a plain box. The plate glass should be ground on one side and polished on the other. The knife is rigidly held in a T-shaped clamp welded to a long arm. The arm is fastened to a movable upright support by means of a utility buret clamp, adjusted to allow free vertical movement only. The general arrangement of the apparatus is shown in Figure 1. A counterweight may be attached to the arm to reduce pressure of the knife edge on the polishing-grinding surface.

The angle of the bevel may be calculated and adjusted from the right-angle triangle ABC in Figure 1. By moving the vertical support, the direction of movement of the glass surface across the knife edge may be varied (Fig. 2).

For coarse grinding, the ground side of the glass plate should be used with coarse abrasive (Aloxite 500, 20 micron size). For fine grinding, the polished surface with fine abrasive (Linde Alumina "B", 0.1 micron size) should be used. For polishing, use fine abrasive. The knife position should be altered so that the glass surface rotates away from the knife edge. (Fig. 2, positions A and B). Stropping is not necessary.

For the small laboratory, operating on a limited budget, the complex mechanical sharpeners are prohibitively costly. This simple sharpener, using the ground glass principle, was assembled at a cost of less than $35.00. It has produced consistently sharp and durable knife edges.

* Received for publication, July 11, 1949.

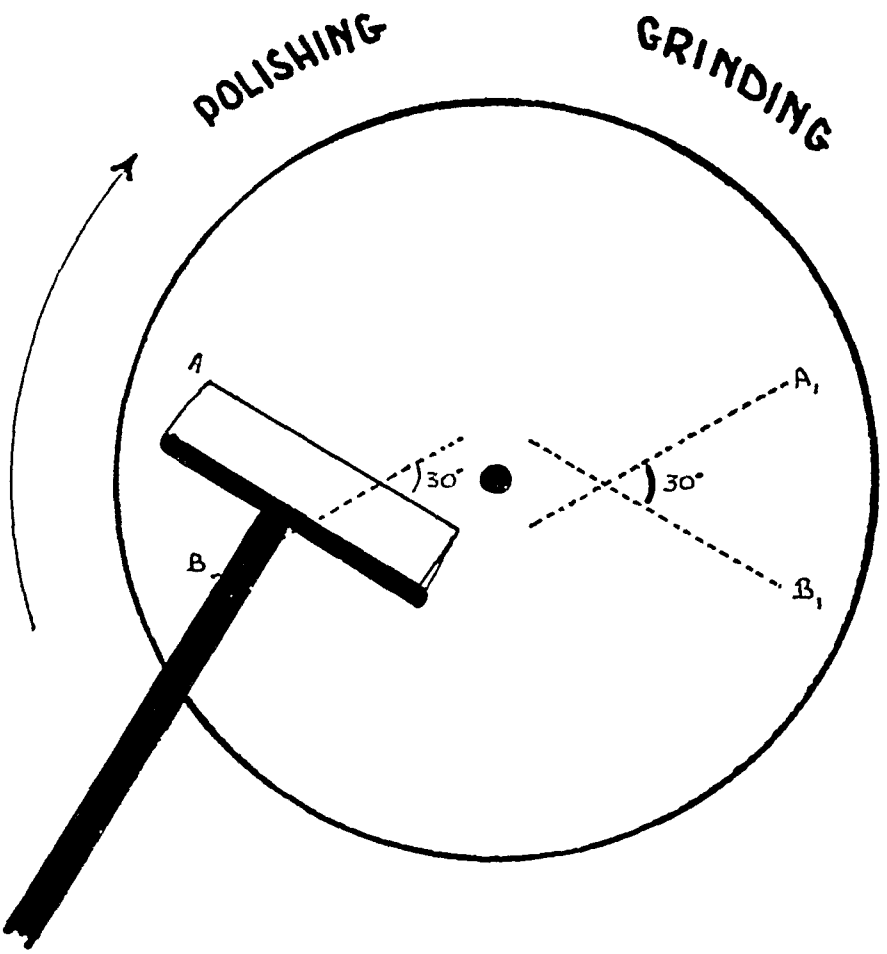


FIG. 2. Alternative positions of knife edge on rotating glass surface during grinding and polishing

REFERENCES

1. JOHNSTON, F. J.: A rapid glass plate method for sharpening microtome knives. Canad. J. Med. Tech., **10**: 157–162, 1948.
2. RICHARDS, O. W.: The Effective Use and Proper Care of the Microtome. Buffalo, N. Y.: Spencer Lens Co., pp. 19 and 28, 1942.

INDEX OF SUBJECTS

Acid-fast bacilli
 concentration of, 269
 in sputum, 688
Adsorption levels of prothrombin-free plasmas, 1071
Antibodies, titration of, 265
Apparatus
 instrument to level tissue in paraffin embedding, 897
 laboratory water connections, 499
 microdetermination of cholesterol, 1076
 multiple puncture for vaccination, 492
 paraffin tissue-block trimmer, 1070
 pipetting instrument for serologic use, 693
 prism mirror, 96
 sedimentation tube support, 298
 serum total base and sodium, 659
 sheep blood, collection, 496
 tissue dehydrating and embedding, 1061
Argyrosis, excretion of silver in, 668

Bacteriology
 blood culture bottle, 697
 Clorox and trisodium phosphate, for acid-fast bacilli in sputum, 688
 colored cotton plugs, 98
 concentrating acid-fast bacilli, 269
 divided culture plate, 289
 Dubos' medium, 67
 Maxted's filtrate, 880
 M. tuberculosis from sputum or gastric washings, 91
 penicillin-resistant staphylococci, 1083
 primary isolation of dermatophytes, 95
 solid-and-liquid medium, 697
 streptomycin sensitivity of *M. tuberculosis*, 67, 76
 trichrome stain for culture or smear, 898
 tubercle bacilli, gastric juice, 685
BCG vaccination, apparatus, 492
Bile, bromsulphalein in presence of, 699
Biochemistry
 bromsulphalein in presence of hemolysis, turbidity and bile, 699
 bromsulphalein tests, error in, 700
 co-thromboplastin assay, 861
 flame photometry, 461
 Folin-Ciocalteu's phenol reagent, 297
 microdetermination of cholesterol, 1076
 nomograph for icterus index, 881
 para-aminosalicylic acid blood levels, 886
 protein in edema fluid, 488
 prothrombin-free plasmas, electrophoretic analysis, 1071
 prothrombin, two-stage method, 471
 serum total base and sodium, 659
 silver, excretion of, in argyrosis, 668
 thromboplastin from rabbit lung, 71
 urinary sugar test for blind, 509
 Xenopus laevis for pregnancy tests, 284
Biopsy
 use of sponge in microtechnic, 92, 891
Blood coagulation, co-thromboplastin, 861
Blood culture bottle, use of solid-and-liquid medium, 697
Blood donor sets, silicone-treated needles in, 695
Blood volume, estimation of, 483
Bromsulphalein tests, errors in, 699, 700

Cardiolpin-lecithin, in Kline test, 274
Cerebrospinal fluid, Kline test in, 274
Cholesterol, microdetermination of, 1076
Clorox, acid-fast bacilli from sputum, 688
Collecting multiple blood specimens, 293
Collodial gold reagent, preparation, 86
Concentration of protozoan cysts, 81
Concentrating acid-fast bacilli, 269
Conductivity assembly for serum total base and sodium, 659
Co-thromboplastin assay, in blood coagulation, 861
Cotton plugs, for culture mediums, 98
Culture diagnosis of fungus disease, 865

Dermatophytes, primary isolation of, 95
Dioxane for paraffin embedding, 291
Distribution of erythrocyte fragility, 259
Divided culture plate, 289
Dubos' medium, 67

Electrophoretic analyses, of prothrombin-free plasmas, 1071
Erythrocytes
 fragility, distribution of, 259
 improved suspension medium, 88
 washed and unwashed, in titration of antibodies, 265

Flame photometry, for sodium and potassium in plasma and urine, 461

Fluorescent microscopy, for acid-fast bacilli, 72
Folin-Ciocalteu's phenol reagent, 297
Frozen section technic, 873
Fungi
 culture diagnosis, advances in, 865
 microslide for culture of, 278

Gastric juice, culture of tubercle bacilli from, 91, 685

Hematology
 blood volume, 483
 erythrocyte fragility, distribution of, 259
 Köhler illumination for reticulocytes, 883
 new methylene blue as reticulocyte stain, 895
 photographic recording of hemolysis, 676
 platelets, Texchrome staining of, 681
 sedimentation tubes support, 298
 suspension medium, improved erythrocyte counts, 88
 thin blood films, 98
Hemolysis
 bromsulphalein in presence of, 699
 photographic recording of, 676
Hemolytic streptococci, Maxted's filtrate, 880

Icterus index, nomograph for, 884
Infectious mononucleosis, test for, 665
Iron hematoxylin staining of protozoan cysts, 84

Kline test of cerebrospinal fluid, 274
Knife sharpener, microtome, 1089
Köhler illumination for reticulocytes, 883

Laboratory water connections, 499

Maxted's filtrate, for extracting carbohydrates from streptococci, 880
Metal shadowing, optical microscopy of tissues, 63
Microdetermination of cholesterol, apparatus, 1076
Microslide, for culture of fungi, 278
Microtechnic
 dioxane in dehydration, 291
 frozen section, 873
 instrument to level tissue, 897
 metal shadowing, 63
 paraffin tissue-block trimmer, 1070
 tissue dehydration and embedding, 1061
 use of sponge in biopsy, 92
 use of sponge to obtain gastric tissue, 891
Microtome knife sharpener, 1089
Multiple blood specimens, collection of, 293
Mycobacterium tuberculosis
 from sputum or gastric washings, 91
 streptomycin sensitivity of, 67, 76

New methylene blue, a reticulocyte stain, 895
Nomograph, for icterus index, 884

Optical microscopy of tissues by metal shadowing, 63

Papanicolaou stain for amebae, 81
Para-aminosalicylic acid blood levels, 886
Paraffin tissue-block trimmer, 1070
Parasitology
 Papanicolaou stain for amebae, 81
 protozoan cysts, staining of, 84
Penicillin-resistant staphylococci, 1083
Photographic recording of hemolysis, 676
Pipetting instrument, serologic use, 693
Plasmas, prothrombin-free, 1071
Platelets, Texchrome staining of, 681
Potassium, in plasma and urine, by flame photometry, 461
Pregnancy tests, *Xenopus laevis* in, 284
Primary isolation of dermatophytes, 95
Prism mirror, 96
Protein in edema fluid, 488
Prothrombin, determination of, 471
Protozoan cysts, iron hematoxylin, 84

Rabbit lung, thromboplastin from, 71
Reticulocyte counts, use of Köhler illumination, 883
Reticulocyte stain, new methylene blue, 895
Rh-Hr antibodies in maternal serum, 80

Sedimentation tubes, support for, 298
Serology
 collecting multiple blood specimens, 293
 collodial gold reagent, 86
 infectious mononucleosis, macroscopic test, 665
 Kline test for syphilis, 274
 pipetting instrument, 693
 Rh-Hr antibodies, 80
 sheep blood, collection, 496

silicone-coated needles, 695
silicone-treated test tubes, 296
titration of antibodies, 265
Serum sodium and total base, 659
Sheep blood, apparatus for collection and dispensing, 496
Sheep cells, preservation of, 665
Silicone-coated needles, 695
Silicone-treated test tubes, 296
Silver, excretion of, in argyrosis, 668
Slide-culture technic, streptomycin sensitivity of *M. tuberculosis*, 76
Sodium and potassium in plasma and urine, 461
Solid-and-liquid medium for blood culture, 697
Sponge for biopsy, 92, 891
Sputum, recovery of *M. tuberculosis* from, 91
Staining
iron hematoxylin protozoan cysts, 84
Texchrome silhouette, of platelets, 681
Staphylococci, penicillin-resistant, 1083
Streptomycin sensitivity of *M. tuberculosis*
Dubos' medium, 67
slide-culture technic, 76
Suspension medium for erythrocyte counts 88

Texchrome silhouette staining of platelets, 681
Thin blood films, preparation, 98
Thromboplastin, from rabbit lung, 71
Tissue
dehydrating apparatus, 1061
embedding apparatus, 1061
Trichrome stain, 898
Trisodium phosphate, for acid-fast bacilli in sputum, 688
Tubercle bacilli in gastric juice, 685
Two-stage method for prothrombin, 471

Urinary sugar test, for blind, 500

Vaccination, apparatus for, 492

Water connections, 499

Xenopus laevis, in pregnancy tests, 284

INDEX OF AUTHORS

Abrahamson, E. M., 298, 1076
Armstrong, B. W.: See Wilson, B. J., 886
Aull, J. C., Switzer, P. K., Ownby, F.D., and Milan, J. W., 488

Barr, L. M.: See Ecker, E. E., *et al.*, 259
Batchelor, T. M.: See Boyle, A. J., *et al.*, 461
Bird, E. J.: See Boyle, A. J., *et al.*, 461
Birkhaug, K., 492
Bossak, H. N., 496
Boyle, A. J., Bird, E. J., Jacobson, S. D., Batchelor, T. M., Iseri, L. T., and Myers, G. B., 461
Brecher, G., 895

Cary, S. G.: See Longfellow, D., *et al.*, 880
Cleary, E.: See Turner, R. D., *et al.*, 296
Conger, M. E., 1070
Corper, H. J., and Nelson, C. R., 269
Covey, E. P., 500

Dittebrandt, M., 284

Ecker, E. E., Hiatt, C. W., and Barr, L. M., 259
Edelberg, R.: See Love, W. E., *et al.*, 676
Elton, N. W.: See Giammalvo, J. T., *et al.*, 76

Felsen, J., 289
Fredenburgh, E. J., 86
French, E. M.: See Turner, R. D., *et al.*, 296
Frey, A., and Frey, M., 699
Frey, M.: See Frey, A., 699

Gerbasi, F. S., and Robinson, A. R., 668
Giammalvo, J. T., Natsios, G. A., and Elton, N. W., 76
Gladstone, S. A., 92, 891
Gollub, S., Kaplan, F. E., Meranze, D. R., and Tuft, H., 1071
Greenwalt, T. J., 695
Gurevitch, J., Polishuk, Z., and Hermoni, D., 265

Hazard, J. B., and Stevenson, G. F., 873
Hermoni, D.: See Gurevitch, J., *et al.*, 265

Hiatt, C. W.: See Ecker, E. E., *et al.*, 259
Hosley, R. J., 884
Hunter, C. A., and Victor, F., 293

Iseri, L. T.: See Boyle, A. J., *et al.*, 461

Jacobson, S. D.: See Boyle, A. J., *et al.*, 461
Jernstrom, P., and Perkins, E., 700
Johnson, D. A., 84

Kabler, P., and Lundholm, R., 685
Kaplan, F. E.: See Gollub, S., *et al.*, 1071
Kennedy, J. D., 883
Kline, B. S., and Suessenguth, H., 274
Kuhn, L. R.: See Longfellow, D., *et al.*, 880

Laskaris, T., 95
Lawless, M. C.: See Turner, R. D., *et al.*, 296
Lewis, W. G.: See Tarshis, M. S., 688
Lind, H. E., 72
Lippman, R. W., 499
Littman, M. L., 278
Littman, M. L., McQuown, A. L., and Schneidau, J. D., 865
Lockard, C. D., 91
Lonergan, A. M.: See Rice, W. G., 1033
Longfellow, D., Cary, S. G., and Kuhn, L. R., 880
Love, W. E., Edelberg, R., and MacNichol, E. F., 676
Lundholm, R.: See Kabler, P., 685

MacNichol, E. F.: See Love, W. E., *et al.*, 676
Mann, F. D., 861
Markell, E. K.: See Michael, P., 81
Marwin, R. M., 697
McQuown, A. L.: See Littman, M. L., *et al.*, 865
Meranze, D. R.: See Gollub, S., *et al.*, 1071
Michael, P., and Markell, E. K., 81
Milan, J. W.: See Aull, J. C., *et al.*, 488
Milligan, M., 897
Mollov, M., and Oshinsky, A., 67
Mosher, R. E., 461
Myers, G. B.: See Boyle, A. J., *et al.*, 461

Natsios, G. A.: See Giammalvo, J. T., *et* 76
Nelson, C. R.: See Corper, H. J., 269
Nichols, J., 96

Oshinsky, A.: See Mollov, M., 67
Ownby, F. D.: See Aull, J. C., *et al.*, 488

Perkins, E.: See Jernstrom, P., 700
Polishuk, Z.: See Gurevitch, J., *et al.*, 2

Rappaport, F., and Skariton, M., 88, 66
Rice, W. G., 1089
Rice, W. G., and Lonergan, A. M., 1083
Robinson, A. R.: See Gerbasi, F. S., 668
Rosbash, D. O., 297

Sano, M., 898
Scher, Y., 71
Schneidau, J. D.: See Littman, M. L., *et* 865
Scott, D. B., and Wyckoff, R. W. G., 63
Seegers, W. H.: See Ware, A. G., 471
Skariton, M.: See Rappaport, F., 88, 66
Slattery, M., 80
Stevenson, G. F.: See Hazard, J. B., 87
Strumia, M. M., Wall, R., Strumia, P. 483
Strumia, P. V.: See Strumia, M. M., 48
Suessenguth, H.: See Kline, B. S., 274
Sunderman, F. W., 659
Svedmyr, A., 693
Switzer, P. K.: See Aull, J. C., *et al.*, 48

Tannenberg, J., 1061
Tarshis, M. S., and Lewis, W. G., 688
Tuft, H.: See Gollub, S., *et al.*, 1071
Turner, R. D., Lawless, M. C., Cleary, and French, E. M., 296

Victor, F.: See Hunter, C. A., 293
Vroman, L., 681

Walker, T. F., 291
Wall, R.: See Strumia, M. M., *et al.*, 483
Ware, A. G., and Seegers, W. H., 471
Wilson, B. J., and Armstrong, B. W., 88
Wyckoff, R. W. G.: See Scott, D. B., 63

TECHNICAL BULLETIN

of the

REGISTRY OF MEDICAL TECHNOLOGISTS

Editor

S. E. GOULD

Wayne County General Hospital, Eloise, Michigan

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON, Milwaukee, Wis.
J. J. ANDUJAR, Fort Worth, Texas
J. E. ASH, Washington, D. C.
L. BERMAN, Detroit, Mich.
G. R. BISKIND, San Francisco, Calif.
O. A. BRINES, Detroit, Mich.
I. DAVIDSOHN, Chicago, Ill.
E. A. GALL, Cincinnati, Ohio
W. M. GERMAIN, Cincinnati, Ohio
A. S. GIORDANO, South Bend, Ind.
G. GOMORI, Chicago, Ill.
F. W. HARTMAN, Detroit, Mich.
J. W. KERNOHAN, Rochester, Minn.
J. A. KOLMER, Philadelphia, Pa.
H. J. LINN, Dearborn, Mich.
T. B. MAGATH, Rochester, Minn.
A. R. MORITZ, Boston Mass.
S. P. REIMANN, Philadelphia, Pa.
S. B. ROSE, Philadelphia, Pa.
A. H. SANFORD, Rochester, Minn.
E. W. SCHULTZ, Palo Alto, Calif.
F. W. SUNDERMAN, Houston, Texas
W. S. THOMAS, Rochester, N. Y.
E. WITEBSKY, Buffalo, N. Y.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. MONTGOMERY, Muncie, Ind.
A. G. FOORD, Pasadena, Calif.
F. B. LYNCH, Philadelphia, Pa.
F. B. QUEEN, Portland, Ore.
R. G. STILLMAN, New York, N. Y.
A. H. WELLS, Duluth, Minn.

Volume 19

MUNCIE, INDIANA

1949

TABLE OF CONTENTS

Number 1, January 1949

Metal Shadowing for the Optical Microscopy of Certain Tissues. D. B. Scott and R. W. G. Wyckoff 63
Value of Dubos' Medium in Tests for Streptomycin Sensitivity of *Mycobacterium tuberculosis*. M. Mollov and A. Oshinsky 67
Preparation of Thromboplastin from Rabbit Lung. Y. Scher 71
Limitations of Fluorescent Microscopy for Detection of Acid-Fast Bacilli. H. E. Lind. 72
Streptomycin Sensitivity of *Mycobacterium tuberculosis*. A Slide-Culture Technic. J. T. Giammalvo, G. A. Natsios and N. W. Elton 76
Testing for Rh-Hr Antibodies in Maternal Serum. A Satisfactory Source of Erythrocytes. M. Slattery 80
Modified Papanicolaou Stain for Amebae. P. Michael and E. K. Markell 81
Concentration of Protozoan Cysts for Iron Hematoxylin Staining. D. A. Johnson 84
Preparation of Colloidal Gold Reagent. E. J. Fredenburgh 86
An Improved Suspension Medium for Estimation of Erythrocyte Counts. F. Rappaport and M. Skariton 88
Recovery of *Mycobacterium tuberculosis* from Sputum or Gastric Washings. Use of 250 ml. Centrifuge Bottle. C. D. Lockard 91
Use of Sponge in the Collection and Preparation of Tissue for Biopsy. S. A. Gladstone 92
A Simple Efficient Method for Primary Isolation of Dermatophytes. T. Laskaris 95
An Economical Prism Mirror. J. Nichols 96
Technical Suggestions 98
Notice 98

Number 2, March 1949

A Method of Determining the Distribution of Erythrocyte Fragility in Human Blood. E. E. Ecker, C. W. Hiatt and L. M. Barr 259
Titration of Physiologic and Immune Antibodies with Washed and Unwashed Erythrocytes. J. Gurevitch, Z. Polishuk and D. Hermoni 265
Methods of Concentrating Acid-Fast Bacilli. H. J. Corper and C. R. Nelson 269
Standard Kline Test of Cerebrospinal Fluid for Syphilis, Employing Cardiolipin-Lecithin Antigen. B. S. Kline and H. Suessenguth 274
A Special Microslide for Culture of Fungi. M. L. Littman 278
Use of *Xenopus laevis* in Testing for Pregnancy. Analysis of 1100 Tests. M. Dittebrandt 284
The Divided Culture Plate. J. Felsen 289
Dioxane Method of Dehydration of Tissues for Paraffin Embedding. T. F. Walker 291
An Improved Simplified Technic for Collecting Multiple Blood Specimens. C. A. Hunter and F. Victor 293
Use of Silicone-Treated Test Tubes to Obtain Serum from Blood. R. D. Turner, M. C. Lawless, E. Cleary and E. M. French 296
Preparation of Folin-Ciocalteu's Phenol Reagent. Using 30 Per Cent Hydrogen Peroxide. D. O. Rosbash 297
A Convenient Support for Sedimentation Tubes. E. M. Abrahamson 298
News and Notices 300

Number 3, May 1949

The Use of Flame Photometry for the Quantitative Determination of Sodium and Potassium in Plasma and Urine. R. E. Mosher, A. J. Boyle, E. J. Bird, S. D. Jacobson, T. M. Batchelor, L. T. Iseri and G. B. Myers 461

Two-Stage Procedure for the Quantitative Determination of Prothrombin Concentration. A. G. Ware and W. H. Seegers 471
A Method for Estimation of Blood Volume. M. M. Strumia, R. Wall and P. V. Strumia 483
Rapid Method for Determining Edema Fluid Proteins. J. C. Aull, P. K. Switzer, F. D. Ownby and J. W. Milan 488
A Detachable Spring-Actuated Multiple Puncture Apparatus for BCG Vaccination. K. Birkhaug 492
An Apparatus for the Aseptic Collection and Dispensing of Sheep Blood. H. N. Bossak 496
Rapid Interchange of Laboratory Water Connections. R. W. Lippman 499
A Urinary Sugar Test for the Blind. E. P. Covey 500
Notice 500

Number 4, July 1949

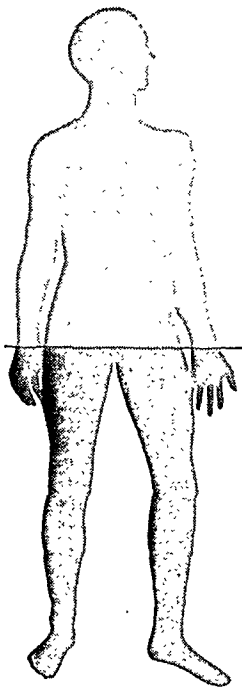
Rapid Measurement of Serum Total Base and Estimation of Serum Sodium. An Improved Conductivity Assembly. F. W. Sunderman 659
Rapid Macroscopic Test for Infectious Mononucleosis. A Method for Preservation of Sheep Cells. F. Rappaport and M. Skariton 665
Excretion of Silver in a Patient with Argyrosis. Ineffectiveness of Treatment with BAL. F. S. Gerbasi and A. R. Robinson 668
Photographic Recording of Hemolysis Using a Portable Electrocardiograph. W. E. Love, R. Edelberg and E. F. MacNichol 676
Texchrome Silhouette Staining of Platelets. Morphology *in Vitro* Before and During Coagulation. L. Vroman 681
Culture of Tubercle Bacilli from Specimens of Gastric Juice. P. Kabler and R. Lundholm 685
Use of Clorox and Trisodium Phosphate in Demonstration of Acid-Fast Bacilli in Sputum. M. S. Tarshis and W. G. Lewis 688
An Accurate Pipetting Instrument for Serologic Use. A. Svedmyr 693
The Use of Silicone-Coated Needles in Blood Donor Sets. T. J. Greenwalt 695
An Improved Blood Culture Bottle Utilizing a Solid-and-Liquid Medium. R. M. Marwin 697
The Determination of Bromsulphalein in the Presence of Hemolysis, Turbidity and Bile. A. Frey and M. Frey 699
A Source of Error in Bromsulphalein Tests. P. Jernstrom and E. Perkins 700

Number 5, September 1949

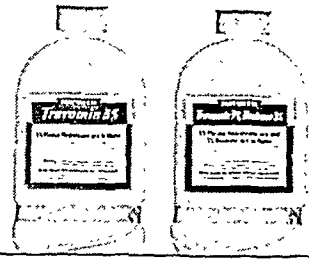
Co-Thromboplastin Assay. A Means of Study of Abnormalities of Blood Coagulation. F. D. Mann 861
Recent Advances in the Culture Diagnosis of Fungus Disease. M. L. Littman, A. L. McQuown and J. D. Schneidau 865
A Frozen Section Technic. J. B. Hazard and G. F. Stevenson 873
Use of Maxted's Streptomyces Filtrate for Rapidly Extracting Group Carbohydrates from Hemolytic Streptococci. D. Longfellow, S. G. Cary and L. R. Kuhn 880
Use of Köhler Illumination for Reticulocyte Counts. J. D. Kennedy 883
Nomograph for Rapid Calculation of Icterus Index When Determined by Colorimeter. R. J. Hosley 884
Determination of Para-Aminosalicylic Acid Blood Levels. B. J. Wilson and B. W. Armstrong 886
Use of Sponge in Obtaining Tissue from Stomach for Biopsy. S. A. Gladstone 891
New Methylene Blue as a Reticulocyte Stain. G. Brecher 895
An Instrument to Level Tissue in Paraffin Embedding. M. Milligan 897
Trichrome Stain for Tissue Section, Culture or Smear. M. Sano 898

NUMBER 6, NOVEMBER 1949

Automatic Tissue Dehydrating and Embedding Apparatus. J. TANNENBERG 1061
Paraffin Tissue-Block Trimmer. M. E. CONGER 1070
Critical Adsorption Levels and Electrophoretic Analyses of "Prothrombin-Free" Plasmas. S. GOLLUB, F. E. KAPLAN, D. R. MERANZE AND H. TUFT 1071
An Improved Apparatus for the Microdetermination of Cholesterol. E. M. ABRAHAMSON 1076
Penicillin-Resistant Staphylococci. W. G. RICE AND A. M. LONERGAN 1083
An Improvised Microtome Knife Sharpener. W. G. RICE 1089
Index of Subjects, Volume 19 1091
Index of Authors, Volume 19 1093
Table of Contents, Volume 19 iii



a valuable aid



in postsurgical recovery

A large percentage of patients enter surgery at a low plasma protein level, and the operative procedure makes further inroads on the already depleted protein reserves.[1,2] A lowered protein level is unfavorable to recovery. It predisposes the patient to pulmonary edema and infection, retarded wound and fracture healing and impaired liver function.[3,4] A high protein level is conducive to rapid healing.[5]

Since diets immediately following surgery are usually inadequate, protein digests given intravenously result in improved strength, appetite, and wound healing. Consequently, they greatly accelerate recovery.[6,7]

2000 cc. of TRAVAMIN 5% a day will satisfy the protein requirements of a high percentage of surgical patients. 2000 to 4000 cc. a day are given according to requirements.[8] TRAVAMIN is made from bovine plasma.

TRAVAMIN 5% IN WATER
TRAVAMIN 5%, DEXTROSE 5% IN WATER

bibliography

1. WHITE, C. S.: "Intravenous Fluids with Particular Reference to Amino Acids," M. Ann. District of Columbia, 15:251 (June) 1946

2. MEYER, J. A., KOZOLL, D. D.: "Protein Deficiency in Surgical Patients," Surg., Gyn. & Obst., 78:181 (February) 1944

3. GLENN, F., *et al:* "Use of Protein Hydrolysates —Conference on Therapy," Am. J. Med., 3:472 (October) 1947

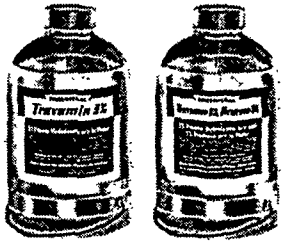
4. CASTEN, D., BODENHEIMER, M., BARCHAM, I.: "A Study of Plasma Protein Variations in Surgical Patients," Ann. Surg., 117:52 (January) 1943

5. BAILEY, H.: Surgery of Modern Warfare, Baltimore, Williams and Wilkins Co., 1941, p. 847

6. DAVIS, H. H.: "Amino Acid Intravenously in Surgical Patients," Nebraska Med. Journal, 30:51 (February) 1945

7. CANNON, P. R.: "Protein Metabolism and Resistance to Infection," J. Mich. M. Soc., 43:323 (April) 1944

8. WEINSTEIN, J. J.: "Intravenous, Subcutaneous and Rapid Intramuscular Infusions of 'Protein Hydrolysate'," Surg., Gyn. & Obst., 87:93 (July) 1948



PARENTERAL **Travamin*** 5% PLASMA HYDROLYSATE

* *formerly PROTEIN HYDROLYSATE, BAXTER*

Product of
BAXTER LABORATORIES, INC.
Morton Grove, Illinois

Distributed and available only in the 37 states east of the Rockies (except in the city of El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION

GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

for

BLOOD TYPING
RH TESTING

you need serum of the highest titer available.

ANTI-HUMAN SERUM (rabbit)
for the Anti-Globulin Test (Coombs')

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human Red Cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic Serum
for control tests

P.S.A.
(Paraffin Section Adhesive)

Write for complete information.

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York



(In writing to advertisers, please mention the Journal—it helps.)

About to Appear

STRESS

Approximately, 800 pages. Extensively illustrated in a beautiful and sturdy binding. Price: $14.00

Under the title "STRESS", Professor Hans Selye wrote an extensive monograph describing his investigations on the *"alarm-reaction"*, the *"general-adaptation-syndrome"* and the *"diseases of adaptation"* (especially rheumatic, renal and cardiovascular diseases), correlating personal observations with the extensive pertinent world-literature.

This book is expected to appear towards the end of 1949, until which date it may be ordered at a $3.00 discount, that is for $11.00, if payment is made with orders addressed directly to us. This will also assure delivery as soon as printing is completed.

Second Edition 1949

Two other books by the same author:

TEXTBOOK OF ENDOCRINOLOGY

916 pages. Illustrated and bound in heavy duty Fabrikoid. Price: $13.50

REVIEWS

AMERICAN JOURNAL OF DIGESTIVE DISEASES: "It is perhaps impossible to render the slightest adverse criticism of this volume, inasmuch as it completely serves the purpose for which it was written."

ANNALS OF INTERNAL MEDICINE: "No serious student of endocrinology can afford to do without this work."

JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION: "The book is excellently printed on good paper, and the illustrations are among the finest available in any of the books devoted to such subjects. The volume avoids the difficulties associated with the use of other textbooks in the field, of too much pro and con discussion, by a didacticism which represents the voice of authority. Nevertheless, the author does not hesitate to indicate the lack of positive information where it is not yet available."

JOURNAL OF PATHOLOGY: "Without doubt, this book will be of real value to clinicians, pathologists and students alike."

QUARTERLY REVIEW OF PEDIATRICS: "The pediatrician will find more of what he wants to know in this book than in any other known to the reviewer."

OVARIAN TUMORS

(An independent section of the "ENCYCLOPEDIA OF ENDOCRINOLOGY") 2 volumes, 776 pages, 11¾ x 10 inches. Sturdily bound in loose-leaf style. Illustrated. Price: $22.75

All prices include mailing charges, but for shipments outside Canada, payment must be made in U.S. Currency or its equivalent in Canadian dollars.

For expediency, place orders directly with Acta Endocrinologica Inc., 5465 Decarie Blvd., Montreal 29, Canada.

Advertisers in the Journal Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.

FOR BEST RESULTS USE

USSLA Products

KRAUTMAN'S STABLE BUFFERED STARCH AND SPECIAL DEPROTEINIZING REAGENT are ready at all times for the *immediate, accurate,* and *simple* analysis of **BLOOD AMYLASE.** Reagents for 65 determinations.....$4.75

KRAUTMAN'S DEPROTEINIZING REAGENT SALT is used to make a stable reagent for the *preparation of protein-free blood filtrates* for such determinations as Urea N, Glucose, Uric Acid, and Creatinin,

One bottle for 2 liters of solution..... $2.50
Five btls., each for 2 l. of solution.....$9.35

NESSLER'S 'IODIZED' SALT is *ideal* for the preparation of Working Nessler's Solution. This solution is *GUARANTEED* to give *accurate Results* in the determination of Urea N in Blood and Urine.

68 Gm. for 1 liter of solution.....$1.90

U. S. Scientific Laboratories

237 Christopher Ave. Brooklyn 12, N. Y.

we say they're better

and so will you when you've

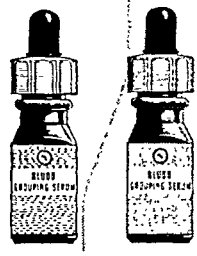
proved it in your own laboratory

HYLAND BLOOD GROUPING SERUM

Anti-A and Anti-B

Prepared from carefully selected donors to assure a highly potent, rapid acting, and specific product. Accurate results with maximum speed.

Available in 2 cc. and 5 cc. sets.



HYLAND ANTI-Rh SERUMS

Anti-Rh_0 (Anti-D) Typing Serum

Anti-Rh_0 rh' rh" (Anti-CDE) Typing Serum

Outstanding serum for slide method—rigidly tested for titer, avidity, specificity and sterility. Prepared from selected, immunized donors.

Both types available in 2 cc. and 5 cc. sizes.



make this test at our expense!

Clip the coupon for your free samples today! You'll receive generous samples of Rh_0 Typing Serum and Blood Grouping Serum. Enough to convince you that their speed, accuracy and titer are unsurpassed.

Hyland Laboratories

4534 Sunset Boulevard, Los Angeles 27, California

Please send me generous samples of Rh_0 and Blood Grouping Serums.

Your Name_____

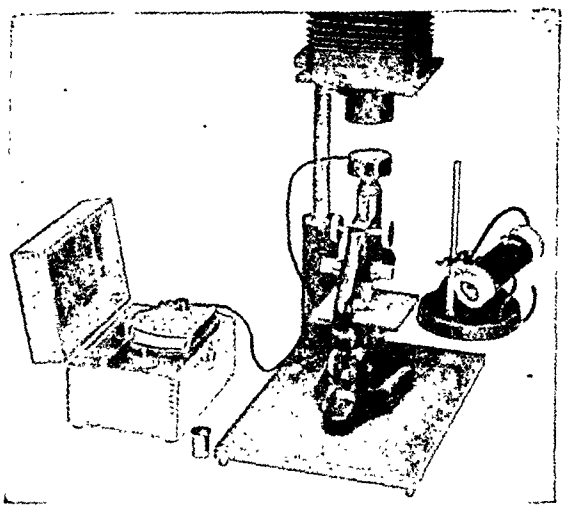
Hospital or Lab. Name_____

Address_____

City and State_____

From what dealer do you regularly buy your typing serums?_____

(In writing to advertisers, please mention the Journal—it helps.)



PHOTOVOLT

Exposure Photometer Mod. 200-M

for

PHOTOMICROGRAPHY

A photoelectric instrument of high accuracy and sensitivity, dependable and simple in operation, designed to measure light at microscope exit for determination of exact exposure time in black-and-white as well as color photomicrography **$65.—**

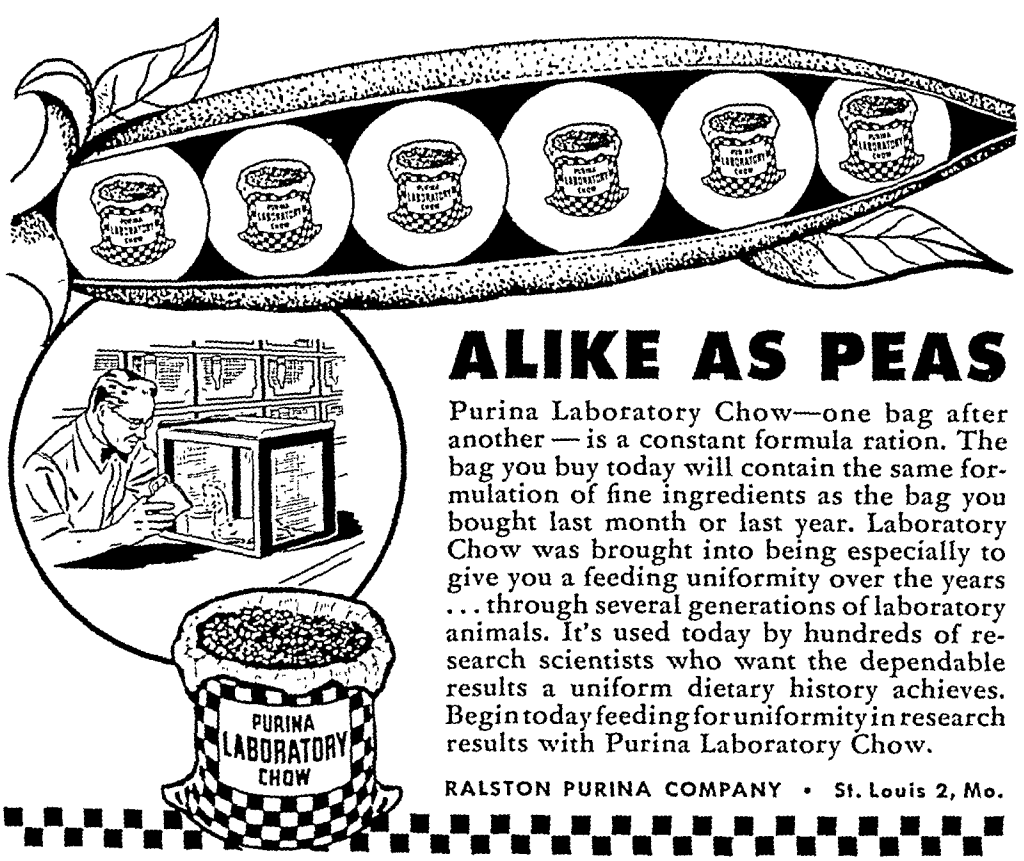
Also: **Electronic Photometers Mod. 500-M and 512-M**

for
- light and color readings on small areas in focal plane
- quantitative histochemical and cytochemical analysis
- measurement of lowest light values with photo-multiplier

Write for Circular #810 and for 22-page Technical Information Bulletin #812

PHOTOVOLT CORP.

95 Madison Ave. — Clinical Colorimeters, Fluorescence Meters, Hemoglobinometers, pH Meters, Densitometers — New York 16, N. Y.



ALIKE AS PEAS

Purina Laboratory Chow—one bag after another — is a constant formula ration. The bag you buy today will contain the same formulation of fine ingredients as the bag you bought last month or last year. Laboratory Chow was brought into being especially to give you a feeding uniformity over the years ... through several generations of laboratory animals. It's used today by hundreds of research scientists who want the dependable results a uniform dietary history achieves. Begin today feeding for uniformity in research results with Purina Laboratory Chow.

RALSTON PURINA COMPANY • St. Louis 2, Mo.



Fisher
ELECTROPHOTOMETER

—eliminates the human factor—

The Fisher Electrophotometer provides accurate, fast and simple analysis for the technician in the busy clinical laboratories of today. Accurate for research, fast for routine or emergency, simple to operate even for the most inexperienced technician, this instrument can be used wherever the color of a solution varies in a definite manner with the concentration of the constituent. It is the standard instrument for clinical colorimetric analysis.

The Fisher Electrophotometer is considerably more sensitive to color, and is more reliable than any technician's eye. Once a calibration curve is made for the standard solutions of any determination, subsequent analyses are conducted as rapidly as the simple operations can be made by the technician. Complete operating instructions and thirteen sample, step-by-step, clinical methods are included in a new 48-page manual furnished free with each instrument.

Fisher Electrophotometer, complete with galvanometer, three filters, three 23-ml. absorption cells, manual of typical procedures for use with 110 volts, 50-60 cycle A.C. only **$210.00**

Headquarters for Laboratory Supplies

FISHER SCIENTIFIC CO.
717 Forbes St., Pittsburgh (19), Pa.
2109 Locust St., St. Louis (3), Mo.

EIMER AND AMEND
Greenwich and Morton Streets
New York (14), New York

In Canada: Fisher Scientific Co., Ltd., 904 St. James Street, Montreal, Quebec

(In writing to advertisers, please mention the Journal—it helps.)

Since 1928

GUINEA PIGS

RABBITS

(Friedman Does)

WHITE MICE

Satisfactorily shipped 1000 miles and more

By

HILLTOP CAVIARY

Box 195 Scottdale, Pa.

Phone 8113R12

★

When writing for quotations or other information, please indicate weight, sex and NUMBER of animals you require.

POLYPHIL
Red Sheep Cells

Uniformity and dependability in the complement-fixation field have long been retarded through the use of variable reagents. Inconsistency in cell resistance has been the cause of many unsatisfactory complement-fixation tests. To help eliminate this condition Carworth Farms is pleased to announce the production of their new and improved POLYPHIL Red Sheep Cells.
Other Serological and bacteriological products available are . . .

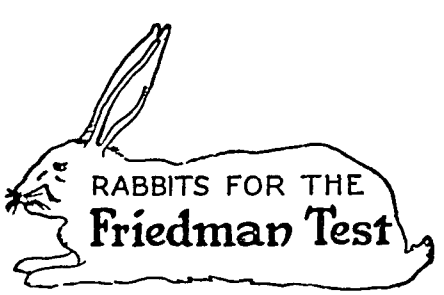
VACSEAL COMPLEMENT
STERILE SHEEP BLOOD • RABBIT BLOOD
STERILE HORSE BLOOD • HORSE SERUM
LYOPHILIZED RABBIT PLASMA

A trial package of any of the above products will be sent to laboratories on request.

CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK



WHITE MICE
WHITE RATS



RESEARCH SUPPLY CO.
2436 W. York Street
Philadelphia 32, Pa.

FUNCTIONAL CARDIOVASCULAR DISEASE

by Meyer Friedman, M.D.

Harold Brunn Institute for Cardiovascular Research, Mt. Zion Hospital, San Francisco

Presents a strong case, backed by physiological evidence, for the theory of Cortico-hypothalamic Imbalance.

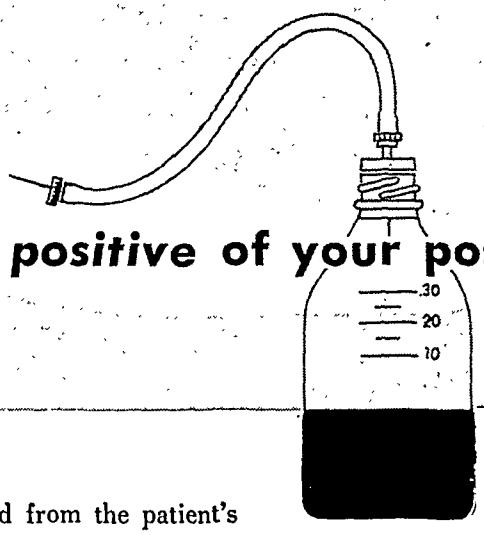
Discusses functional cardiovascular disease as distinguished from organic derangements.

340 pages $3.00

The Williams & Wilkins Company
Baltimore 2, Md.

(In writing to advertisers, please mention the Journal—it helps.)

NOW



you can be *positive* of your positives

with the B-D VACUTAINER® CULTURE BOTTLE

containing Trypticase Soy Broth with CO_2 added

Direct delivery of blood from the patient's vein, through a closed, sterilized system, into Trypticase Soy Broth media in the new B-D VACUTAINER CULTURE BOTTLE . . .

- **Provides *positive* positives and valid negatives**
- **Prevents outside contamination**
- **Avoids incorrect selection and multiplicity of media**

Aerobic or anaerobic conditions may be obtained at time blood specimen is taken. Bottle reaches laboratory ready for immediate incubation . . . speeds laboratory handling . . . provides faster diagnosis. Microaerophiles grow luxuriantly.

Prepared and sterilized by Baltimore Biological Laboratories, Inc., Baltimore, Md.

Write for descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE and learn how easy it is to add this new element of assurance to your blood culture procedures.

BECTON, DICKINSON & CO., Dept. 27-L
15 Ames Avenue,
Rutherford, N. J.

Please send me descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE that takes the guesswork out of blood culture procedures.

NAME_____

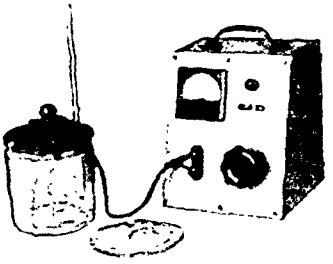
ADDRESS_____

CITY_____ ZONE____ STATE_____

BECTON, DICKINSON & CO., RUTHERFORD, N. J.

(In writing to advertisers, please mention the Journal—it helps.)

Eberbach
BONE DECALCIFIER



Decalcifies bone rapidly by electrolysis. This latest procedure preserves soft tissue patterns and staining properties better than slow acid immersion techniques which require days. Decalcifier, without electrodes, sells for only $75.00. Request Bulletin 180.

Eberbach & Son Company
LABORATORY APPARATUS & SUPPLIES
ANN ARBOR, MICH.



XENOPUS LAEVIS TOAD

Pregnancy Diagnosis

Animal Costs Greatly Reduced

We assure you of a continuous supply. Accurate, speedy and inexpensive. Average animal cost per test, 20 cents to 35 cents. We carry a complete stock of Laboratory Tanks, Isolation Jars, Holders, and correct reagents and apparatus for running tests Speedy delivery via AIR EXPRESS. Current brochure showing new low prices sent upon request.

Jay E. Cook, Importer

P. O. Box 302 Baltimore 3, Md., U.S.A.
Cable Address—XENOPUS-BALTIMORE



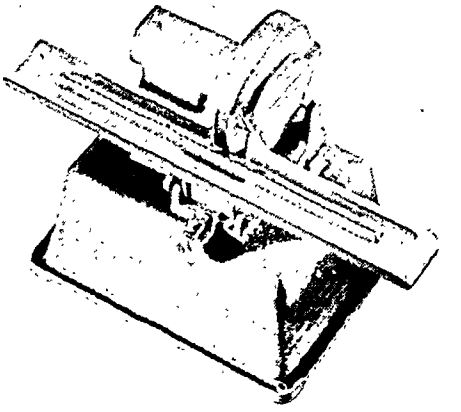
Stryker
AUTOPSY SAW

A new instrument which simplifies bone cutting

Electrically driven, oscillates at high speed to cut bone efficiently with complete safety. Cutting blades do not hurl material. Two-sided blade can be adjusted to three positions. Blade, arbor and shaft are stainless steel. Write Department C for complete information.

ORTHOPEDIC FRAME COMPANY Kalamazoo Michigan

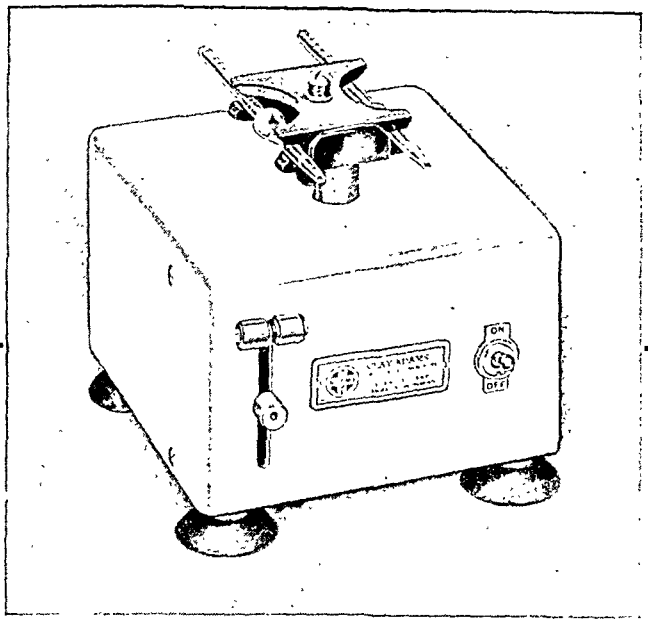
INDISPENSABLE
in your laboratory...



the Berkeley
MICROTOME KNIFE SHARPENER
for perfect edges...three times faster

Operation is simple, eliminates honing and stropping. Assures hollow-ground edges that cut better and last much longer. *Send for our Illustrated Bulletin MKS-1-I*

Berkeley Scientific Company
SIXTH AND NEVIN AVENUE • RICHMOND, CALIF.



for reproducible *blood counts*

YANKEE PIPETTE SHAKER

A precision-built instrument designed to meet all the requirements for proper mixing of blood samples and to give **consistently-uniform blood cell counts.** Using the Yankee Pipette Shaker, repeated tests with the same blood sample will give reproducible results because:

- **The shaker's motion is in one plane only—the vertical; horizontal motion is practically eliminated.**
- **Shaking time is automatically controlled by a built-in adjustable timer.**
- **Complete mixing is obtained in 30 seconds without hemolyzing the blood cells.**

A-2290 Yankee Pipette Shaker for two pipettes; for 120 volts, AC only.....each $45.00



Yankee Pipette Closure

An all-rubber closure that seals the pipette simply and effectively, and protects it from breakage even if it should be dropped.

A-2443 Yankee Pipette Closure.....each $.35
dozen $3.50 gross $35.00

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

Reagents for the Davidsohn Differential Test for Infectious Mononucleosis

BOILED GUINEA PIG KIDNEY ANTIGEN
(Desiccated)

BOILED BEEF CELL ANTIGEN
(Desiccated)

6 Individual Test Ampuls
(0.5 cc each)$4.00
1 cc 1.00
2.5 cc 2.00
5 cc 3.00

SHEEP CELLS
Can be prepared from the following:

WASHED POOLED SHEEP CELLS
Stabilized
A simple dilution prepares the 2% suspension
30 cc $1.50

STERILE SHEEP BLOOD (Alsever's)
(Equal parts of blood and modified Alsever's solution)
15 cc..... $1.50

STERILE SHEEP BLOOD (Citrated)
15 cc $1.50

CAPPEL LABORATORIES
Diagnostic Reagents
Box 352 Wayne, Pa.

BBL

Media for the Cultivation of Brucella

TRYPTICASE SOY BROTH

for *isolation*, especially from blood, and for use wherever a fluid medium is desirable.

TRYPTICASE SOY AGAR

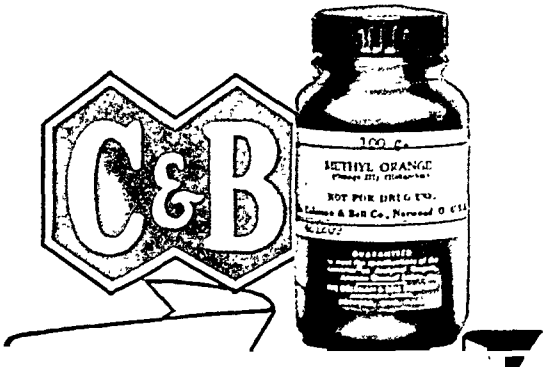
for use with dyes for *identification* of species, and for plate and slant cultures.

CYSTINE TRYPTICASE AGAR

for *maintenance* of stock cultures.

Prices and literature sent on request

BALTIMORE BIOLOGICAL LABORATORY
1640 Gorsuch Avenue Baltimore 18, Md.



Chemical Indicators

Include all of the common indicators used in analytical and biological work, the hydrogen-ion indicators recommended by Sorensen and Clark & Lubs, and in addition many rare indicators suitable for special work. These indicators are available in both the dry form and in solution, ready to use.

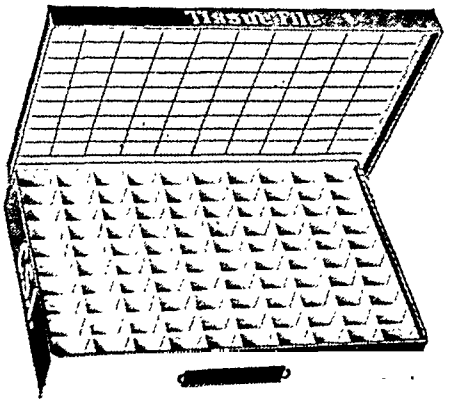
C&B Products are distributed by Laboratory and Physician Supply Houses Throughout the World

Write for copy of our catalog,
The Coleman & Bell Co., Inc., Manufacturing Chemists, Norwood, O., U. S. A.

COLEMAN & BELL

The Cargille

TISSUE FILE

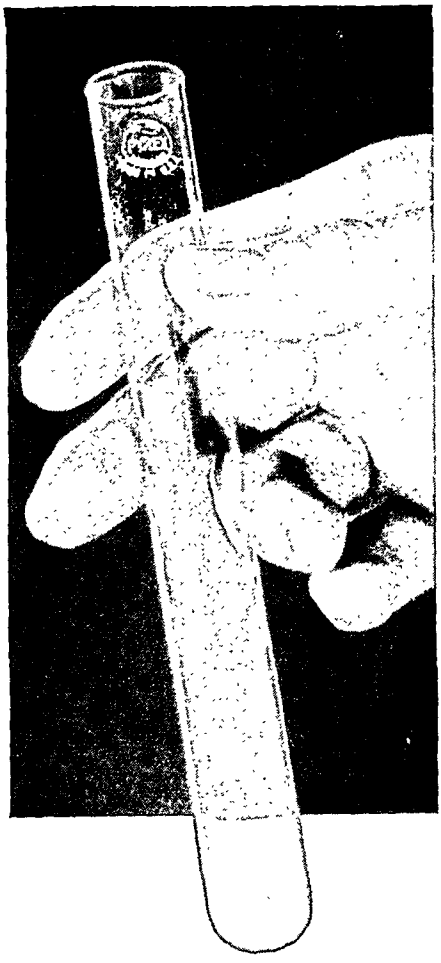


Very convenient for filing and storing Histology Tissues Embedded in Paraffin or Celloidin Blocks.

Write for leaflet TF

CARGILLE SCIENTIFIC INC.
118 Liberty Street New York 6, N.Y.

3½ times the service with



T.M. REG. PYREX U.S. PAT. OFF.

CULTURE TUBES

Clinical research requires culture tubes in large numbers. Consequently, their cost, service life considered, is a matter of importance. From this standpoint alone, you will find that PYREX brand culture tubes are the best investment. Careful records maintained over a period of years by large culture tube users show that PYREX tubes last 3½ times longer than other brands.

This is not so surprising when you consider that no other glass stands up like PYREX brand No. 7740. Compare it with any other laboratory glassware under identical conditions. The superiority of PYREX ware to chemical, thermal and physical resistance will be readily demonstrated.

All of this is reflected in more accurate analysis. Your laboratory dealer carries complete stocks of PYREX brand culture tubes for your ordering convenience.

Stocked by Leading Laboratory Supply Houses

CORNING means research in GLASS

T.M. REG. PYREX U.S. PAT. OFF.

CORNING GLASS WORKS • CORNING, N. Y.

LABORATORY GLASSWARE

TECHNICAL PRODUCTS DIVISION: LABORATORY GLASSWARE • SIGNALWARE • GLASS PIPE • GAUGE GLASSES • LIGHTINGWARE • OPTICAL GLASS • GLASS COMPONENTS

(In writing to advertisers, please mention the Journal—it helps.)

PARASITOLOGY LOAN SETS AVAILABLE

These sets contain a number of slides with mounted specimens and blood smears, as well as vials of suspensions containing various parasitic cysts and ova.

Directors of many Approved Schools have found this material useful in their teaching programs.

A deposit of twenty-five dollars ($25) is necessary. A three-dollar ($3) service charge will be retained and the remainder refunded when the set is returned to the Registry office.

Please direct requests to:

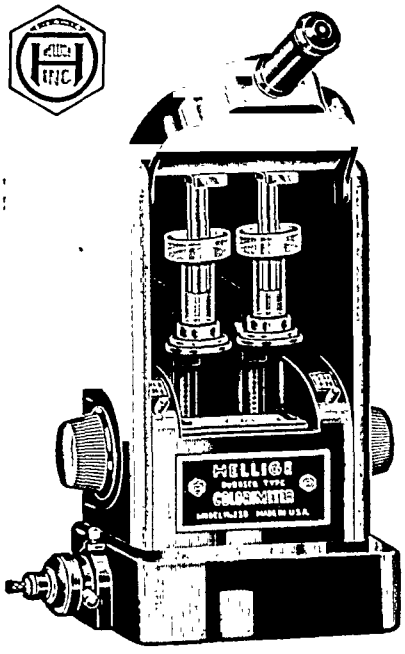
REGISTRY OF MEDICAL TECHNOLOGISTS

of the

American Society of Clinical Pathologists

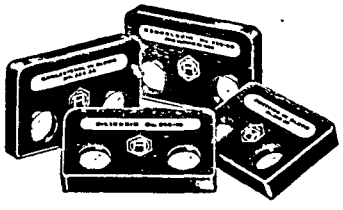
Muncie, Indiana

HELLIGE-DUBOSCQ COLORIMETER



Closely approaching the theoretical ideal, this universal instrument offers the unusual combination of CONVENIENCE, PRECISION AND DURABILITY.





Hellige Non-Fading Glass Color Standards are available for all popular bio-chemical tests. Unlimited in length of service, these permanent standards quickly repay the small original investment by savings in time and chemicals.

Write for Bulletin No. 250

HELLIGE

INCORPORATED

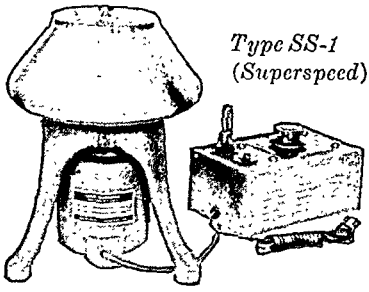
3718 NORTHERN BLVD., LONG ISLAND CITY 1, N.Y.

HEADQUARTERS FOR COLORIMETRIC APPARATUS

SERVALL

ANGLE CENTRIFUGES

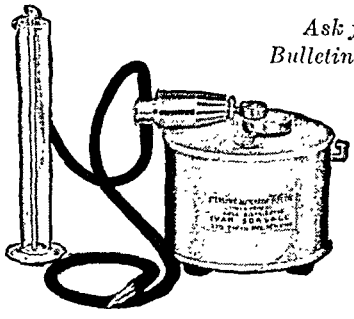
Ask for Bulletin J-111
with complete data on all models



Type SS-1
(Superspeed)

With safety design self-centering device and dynamical balance. 13,000 r.p.m. 20,000 × G. Cap. 8 × 50cc or 15cc tubes.

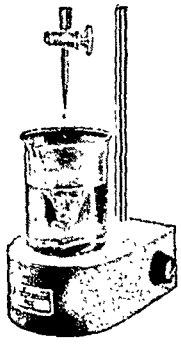
NEW TYPE SS-2 VACUUM CENTRIFUGE
16,000 r.p.m. 45,000 × G.
Capacity 16 × 50cc or 15cc tubes



Ask for Bulletin J-112

STERLING AUTOMATIC
AND
HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.
Easy to Operate—Time Saving



SERVALL

Magnetic Stirrer

Ask for Bulletin J-114

Stirs

- in open vessels
- in closed systems
- under pressure
- under sterile conditions

NEW Thermostatically Controlled Swingable Heating Plate

Novel, shock resistant, Glass encased stirring elements NEW

Manufacturer and Distributor
IVAN SORVALL, Inc.
210 FIFTH AVENUE NEW YORK 10, N.Y.

MEDICAL CLASSICS

SMITH AND GAULT
Essentials of Pathology
(3rd Edition) New

"There is no more suitable text available to the medica student than this interesting, informative and correlative introduction to the essentials of pathology." An "excellent presentation of case histories, textual material and illustrations," reports the *American Journal of Clinical Pathology*. New material has been added, case histories condensed, repetitious material reduced. The ideal book on the subject.

740 Illus., 764 Pages, $12.00

STITT, CLOUGH & BRANHAM
Practical Bacteriology, Hematology & Parasitology

This is a world-famous book, now in its 10th edition. Up-to-date and valuable additions to material, including many new subjects and sections, have been contributed by specialists in their fields; for instance, the new method of preparing colloidal gold solutions now in use at the Naval Medical School Laboratories. There is a summary of the present status of antibiotics . . . and many other informative features. Many new illustrations. The *Review of Gastroenterology* calls it "An indispensable volume for the pathologist and the diagnostician." Order a copy of this classic for personal examination.

765 Illus., 991 Pages, $10.00

Blakiston's New Gould is the only completely new unabridged medical dictionary in 38 years. Do you have one? Textbook edition, $8.50—Thin paper edition, $10.75—Deluxe edition, $13.50.

THE BLAKISTON COMPANY
1012 Walnut Street. Philadelphia 5, Pa.

Please send the items checked. ☐ my check is enclosed; ☐ charge my account It is understood that you will credit my account in full for any books that I wish to return within 10 days.

☐ Smith and Gault's Essentials of Pathology

☐ Stitt, Clough & Branham's Practical Bacteriology, Hematology and Parasitology

☐ Blakiston's New Gould Medical Dictionary ☐ Textbook ☐ Thin-paper ☐ Deluxe

NAME.....ADDRESS

CITY.....ZONE.....STATE

AJCP 11-41

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia...O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Benbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

American Society of Clinical Pathologists

OFFICERS

President: Dr. O. A. Brines, Wayne University College of Medicine, Detroit 26, Michigan
Vice-President: Dr. C. H. Manlove, 5725 S.E. Stark Street, Portland 15, Oregon
President-Elect: Dr. J. B. McNaught, University of Colorado School of Medicine, Denver, Colorado
Secretary-Treasurer: Dr. C. G. Culbertson, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana

STANDING COMMITTEES

EXECUTIVE COMMITTEE

W. S. Thomas, Chairman, Rochester, N. Y.
T. J. Curphey, Long Island, N. Y.
F. W. Konzelmann, Atlantic City, N. J.
L. W. Larson, Bismarck, N. D.
S. P. Reimann, Philadelphia, Pa.
J. R. Schenken, Omaha, Neb.

BOARD OF CENSORS

L. W. Diggs, Chairman, Memphis, Tenn.
J. J. Andujar, Fort Worth, Tex.
F. C. Coleman, Des Moines, Iowa
J. W. Howard, Wilmington, Del.
V. M. Johnson, West Palm Beach, Fla.
C. P. Larson, Tacoma, Wash.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. Montgomery, Chairman, Muncie, Ind.
A. G. Foord, Pasadena, Cal.
F. B. Lynch, Jr., Philadelphia, Pa.
F. B. Queen, Portland, Ore.
R. G. Stillman, New York, N. Y.
A. H. Wells, Duluth, Minn.

APPOINTED COMMITTEES 1948-1949

GENERAL RESEARCH COMMITTEE

M. Strumia, Chairman
B. S. Kline
A. H. Sanford
W. M. Simpson

COMMITTEE ON HEMATOLOGIC TERMINOLOGY

E. E. Osgood, Chairman
L. Berman
F. J. Heck
R. R. Kracke
L. R. Limarzi

BLOOD BANK COMMITTEE

D. H. Kaump, Chairman
L. Y. Dyrenforth
F. W. Hartman
L. W. Larson
R. G. Stillman

COMMITTEE ON REGISTRY OF GENERAL TUMORS

J. W. Kernohan, Chairman
L. V. Ackerman
W. A. D. Anderson
Col. J. E. Ash
S. W. Lippincott
I. Perry

REPRESENTATIVE ON NATIONAL RESEARCH COUNCIL SUBCOMMITTEE ON ONCOLOGY

S. Warren

REPRESENTATIVE ON BOARD OF GOVERNORS OF COLLEGE OF AMERICAN PATHOLOGISTS

T. J. Curphey

COMMITTEE ON SEMINAR

I. Davidsohn, Chairman
P. R. Cannon
Gen. R. O. Dart
J. B. McNaught
R. A. Moore

PROGRAM COMMITTEE

C. G. Culbertson, Chairman
R. A. Moore, Co-Chairman
C. J. Bucher
F. W. Sunderman
F. B. Queen

COMMITTEE ON NECROLOGY

H. R. Brown

COMMITTEE ON EDUCATION AND PUBLICITY

W. O. Russell, Chairman
J. L. Carr
E. F. Hirsch
W. M. Germain
D. A. Nickerson
S. P. Reimann

COMMITTEE TO STANDARDIZE EDUCATION AND TRAINING OF MEDICAL TECHNOLOGISTS

J. J. Andujar, Chairman
F. C. Coleman
O. O. Christianson
R. Miller
E. Moss
M. Oosting
P. Rosenblatt
J. P. Tollman

DIFCO

Isolation and Cultivation of BRUCELLA

Preliminary Enrichment

Isolation of Brucella strains from blood specimens is best accomplished by preliminary enrichment of the sample in medium prepared from Bacto-Tryptose Broth. For isolation from samples of infected milk no enrichment is required.

Isolation of Strains

After enrichment, the blood specimens are streaked on plates of Bacto-Tryptose Agar and the plates are incubated at 37° C. Milk samples are streaked on plates of Bacto-Tryptose Agar prepared with crystal violet to inhibit the streptococci and other Gram-positive organisms.

Differentiation of Types

Bacto-Tryptose Agar to which thionin or basic fuchsin has been added is recommended for the differentiation of newly isolated strains of Brucella.

Mass Cultivation

Bacto-Tryptose Agar, prepared without addition of dyes or other ingredients, supports luxuriant growth of all Brucella. It is an excellent medium for mass cultivation of the organisms for preparation of bacterial vaccines or antigens.

Specify "DIFCO"
THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES

DETROIT 1, MICHIGAN

VOLUME 19 OCTOBER 1949 NUMBER 10

AMERICAN JOURNAL OF CLINICAL PATHOLOGY

OFFICIAL PUBLICATION OF
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R. BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, Indiana University Medical Center, 1040–1232 West Michigan Street, Indianapolis 7, Indiana. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Agents: For the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Australia: Angus and Robertson, Limited, 89–95 Castlereagh Street, Sydney; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 OCTOBER 1949 NUMBER 10

CONTENTS

I. Experimental Cortical Necrosis of Adrenal Gland in the Monkey in Diphtheria and in Malaria. A. GOLDEN AND R. R. OVERMAN..... 899

II. The Effect of Lipo-adrenal Extract on Ionic Balance in Fatal Simian Malaria. R. R. OVERMAN, A. C. BASS, A. K. DAVIS AND A. GOLDEN.. 907

III. Cortical Necrosis of the Adrenal Gland in Man. A. GOLDEN..... 918

Sternal Marrow Studies in Hodgkin's Disease. L. R. LIMARZI AND J. T. PAUL 929

Serum Lipids Following Nephrectomy and in Mercuric Chloride Poisoning. D. H. ATLAS, M. M. CASH AND M. M. KIRSCHEN..... 962

Case Reports

Leiomyosarcoma of the Stomach. R. H. CONLEY AND J. H. OLWIN.... 966

Bone Formation in the Heart. A. J. FINESTONE AND C. F. GESCHICKTER 974

Reticulo-epithelioid Cell Granulomas in Bone Marrow in Herpes Zoster. E. M. SCHLEICHER..... 981

Asymptomatic *Schistosoma japonicum* Infection Associated With Rectal Carcinoma. J. H. HILL..... 985

Editorial: HOWARD T. KARSNER..... 990

Book Reviews..... 992

Books Received..... 998

(In writing to advertisers, please mention the Journal—it helps.)

NATIONAL DIABETES WEEK, OCTOBER 10-16

launches the year-round Diabetes Detection Drive to find the million unknown diabetics in the United States and Canada.

Galatest

The simplest, fastest urine sugar test known

. . . already used by medical groups for diabetes detection in 55 cities.

Galatest is unsurpassed for mass screening tests for urine sugar. During the war it was used to make thousands of urine sugar tests daily at medical centers of the U. S. armed forces. It requires no special laboratory equipment, no test tubes, no boiling. Just one drop of urine, a little Galatest powder, gives color reaction immediately. Galatest is approved by the American Diabetes Association for diabetes detection, and accepted for advertising by The Journal of the American Medical Association.

GALATEST IS READY

for the Diabetes Detection Drive

IN 2 WAYS

1. DOCTOR GUIDANCE

The regular vial of Galatest powder—sufficient for 100 tests—is ideally suited for testing under supervision by officially appointed doctors' committees—in detection centers, industrial medical departments, schools, laboratories, hospital, and physicians offices.

2. *SELF-TESTING*

The new Galatest Unipak—sufficient for 4 tests—for "self-testing-in-the-home" will be made available in drugstores in all areas where local medical groups have approved "self-testing-in-the-home" as part of the national diabetes detection program.

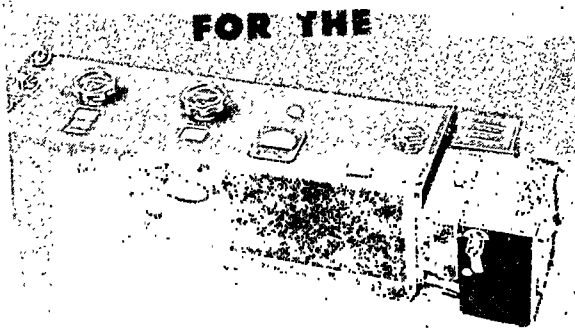
The Denver Chemical Mfg. Co., Inc., manufacturers of Galatest, and Galatest Unipak will cooperate fully with all local, state, or national groups in support of their programs for diabetes detection. Please let us know how we can cooperate with you.

The Denver Chemical Mfg. Co., Inc., 163 Varick Street, New York 13, N.Y.

(In writing to advertisers, please mention the Journal—it helps.)

NEW ACC

FOR THE



BECKMAN "DU" SPECTROPHOTOMETER

Long the standard research spectrophotometer for ultraviolet, visible and near-infrared measurements, the Beckman "DU" assumes even greater usefulness through the development of new accessory equipment.

Most recent additions to the diversified line of accessories are the four described at right. Detailed information on these and other accessories will be gladly sent on request. *Beckman Instruments, National Technical Laboratories, South Pasadena 44, Calif.*

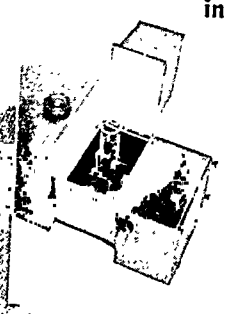
BECKMAN INSTRUMENTS
control modern industries

Radiation Meters—Special Analytical Instruments

pH Meters and Electrodes—Spectrophotometers

TEST TUBE HOLDER

Simplifies direct measurement of samples in round test tubes



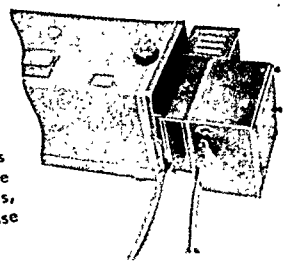
A metal carriage rigidly holds test tubes from 10 mm to 25 mm (1") in diameter in special lightproof housing. A single holder accommodates the various sizes of test tubes and allows exact relocation of tubes horizontally, vertically and rotationally. An accessory unit also permits use of 2" beakers interchangeably in the same carriage.

Designed for oil chemistry; Vitamin "A" analyses; clinical and medical applications, and many other routine uses.

THERMOSPACER

Enables temperature control of sample compartment

A corrosive-resistant, light-tight, metal plate contains internal coils for circulating constant temperature fluid. Thermospacers mount directly on standard instrument. Can be used singly or in combination to maintain sample compartment constant at any temperature between 0 and 100°C. The standard Model DU cell compartment can be held to less than one degree change for a ten degree change in ambient temperature.

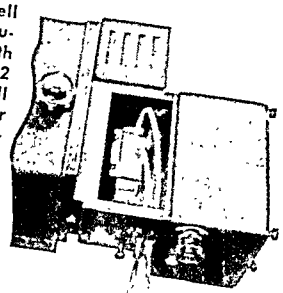


Designed for greatest measurement accuracy with the Model DU . . . and for special measurements such as chemical analyses at constant temperature; the study of reaction rates at various temperatures; precise measurement of extinction values, and other applications where close temperature control is essential.

SHORT PATH FLOW CELL

For extremely short path length measurements on flowing or static samples

Consists of two flat, transparent plates which form an internal cell through which sample can be circulated without exposure to air. Path lengths from 0.05 to 2.5 mm (0.002 to 0.098 in.) available. A special cell compartment containing cell holder and precision positioning carriage attaches directly to Model DU.



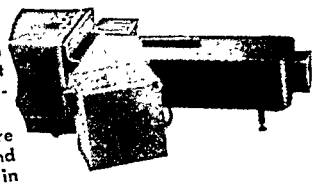
Quartz optics permit full-range measurements, 220 to 1000 mmu . . . All critical surfaces optically polished to ¼ wavelength for precise measurements . . . Extreme flatness of plates allows fluid-tight seal without gaskets or sealing compounds.

Designed for flowing or static measurements on strongly absorbing samples without dilution; on blood samples *in vivo* under anaerobic conditions (such as determining oxygen capacities of whole blood); for microchemical studies of reaction rates, etc.

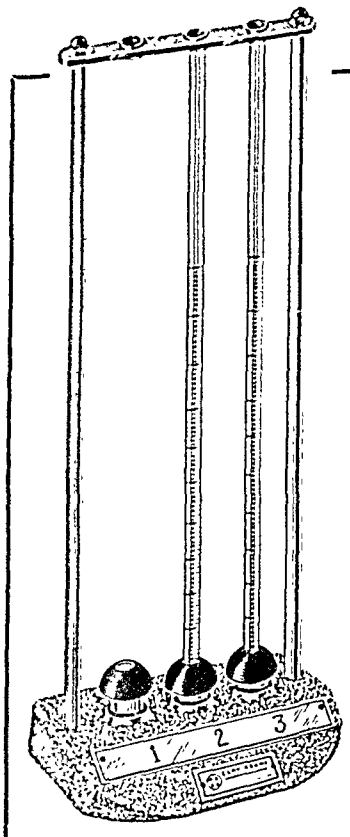
50 cm. LIQUID ABSORPTION CELL

For long path length measurements on weakly absorbing samples

To conserve sample volume, the cell is only 25 cm. long, the light beam passing twice through the sample before reaching the phototube. A special lightproof cell compartment provides a convenient means of standardization without the necessity of using a reference cell. All optical parts are of quartz to utilize full Model DU measurement range. Liquid seals made without gaskets to avoid sample contamination. Careful design eliminates scattered light effects.



Designed for dilute solutions and weakly absorbing samples where a long path length is required. Also for use with flowing samples and for utmost sensitivity in nephelometric measurements to determine, in addition to intensity, the distribution and average size of light-scattering particles.



A-2481

Improved Westergren Blood Sedimentation Apparatus—with new airtight closure

- Improved base mechanism with smoother spring action and rubber spring adapters—insures airtight closure . . . permits easy, one-hand introduction and removal of pipettes.
- Rack can be cleaned more easily because rubber spring adapters keep spilled blood from entering the spring mechanism.
- Rack is sturdily constructed and carefully aligned so that pipettes will be vertical when rack is on a level surface.
- Pipette holders are clearly numbered for ease in identification.

Physician's Outfit

A-2480 Westergren Blood Sedimentation Rack, 3 unit, with 3 pipettes
No. A-2482 **per outfit $12.00**

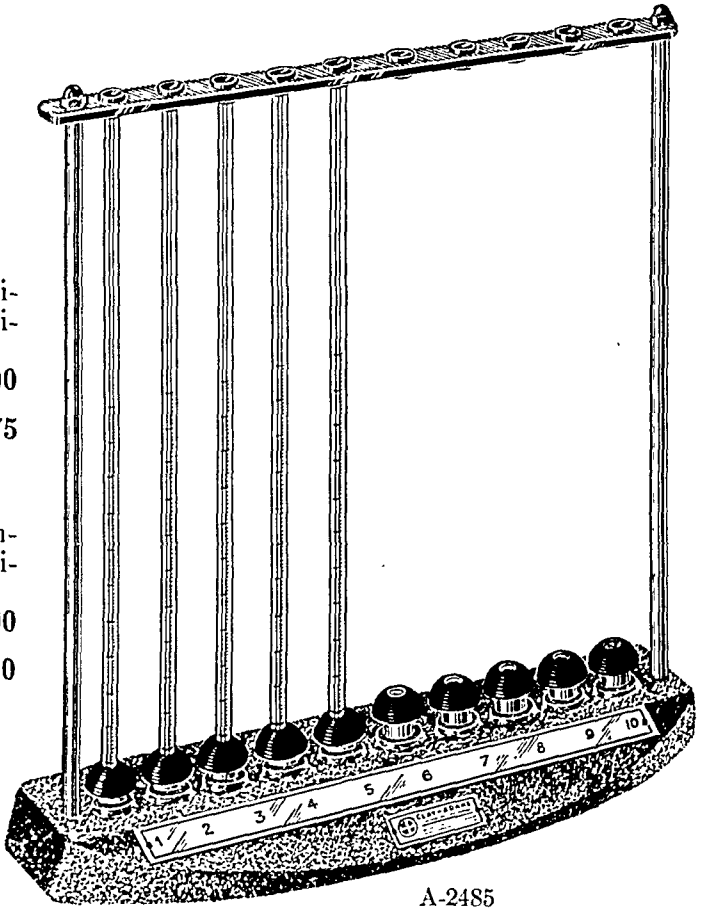
A-2481 Rack, 3 unit **each $9.75**

Hospital Outfit

A-2486 Westergren Blood Sedimentation Rack, 10 unit, with 10 pipettes
No. A-2482 **per outfit $25.00**

A-2485 Rack, 10 unit . . **each $18.00**

Write for Circular 448CP, Sedimentation Apparatus



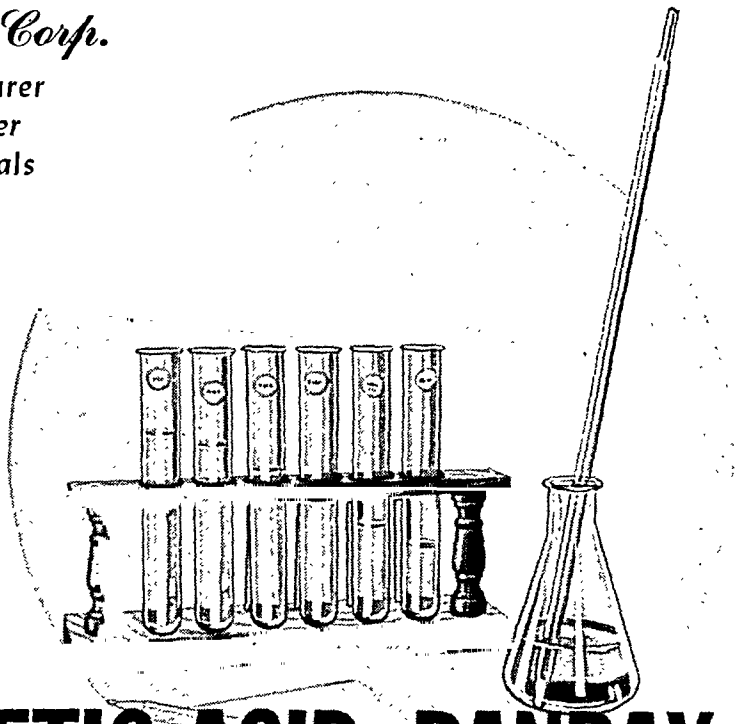
A-2485

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

(In writing to advertisers, please mention the Journal—it helps.)

The Panray Corp.

pioneer manufacturer of DOPA and other specialized chemicals for investigation and research...



announces the availability of...

IODOACETIC ACID, PANRAY

TWICE RECRYSTALLIZED, SPECIAL REAGENT GRADE

for the Iodoacetate Index Determination of Serum Proteins

DOPA "PANRAY"

(3,4-dihydroxyphenylalanine, C. P.)

available in l and dl forms

Synthetic chemical reagents ... capable of revealing the presence of an oxidase in cells elaborating melanin. Valuable indicators of active melanogenesis.

Iodoacetic acid is a recently reported reagent for the determination of *deficient thermal coagulation of serum proteins* in cancer and other pathological conditions.

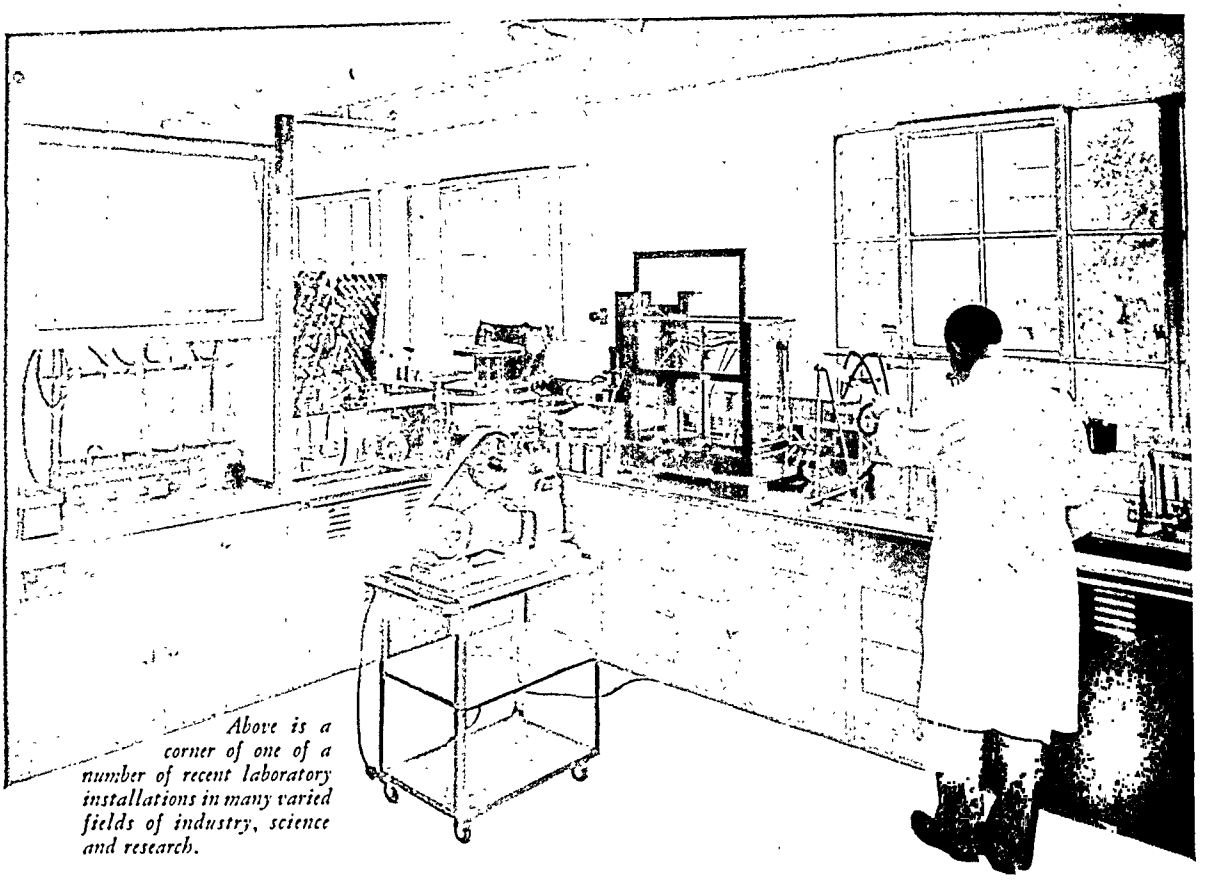
Originally reported by C. Huggins, M.D., G. M. Miller, M.D., and E. V. Jensen, Ph.D.: Thermal Coagulation of Serum Proteins—II. Deficient Coagulation in Cancer and the Iodoacetate Index—*Cancer Research, Vol. 9, No. 3, March 1949.*

Write for prices and other pertinent data

The Panray Corp.

398 BROADWAY
NEW YORK 13, N. Y.

Custom Manufacturers of Fine Organic Chemicals



Above is a corner of one of a number of recent laboratory installations in many varied fields of industry, science and research.

you can save by using a. s. aloe

as your complete source for laboratory supplies

Whether you are expanding, buying replacement equipment, or planning a new laboratory, one complete source for your laboratory supplies saves you *dollars* through lower prices on quantity purchases . . . *time* in placing numerous orders . . . *manpower* in handling multiple shipments.

Furniture, Apparatus, Chemicals

Our complete stocks are geared to all your laboratory needs. Aloe's new stainless steel Moduline Furniture has already proved exceptionally satisfactory for the modern laboratory. Built in standard units, this furniture incorporates the savings of mass production with maximum versatility for long range planning.

Our apparatus stocks include a wide selection of items for routine and special work. Inorganic and organic chemicals, rare sugars, culture media, dyes and stains are always in stock.

Service Departments

We maintain a Planning Department for working up plans on new installations and a Research Laboratory to help locate unusual items or procedures, and in preparing special reagents. Both at your service without charge.

Complete Catalogs

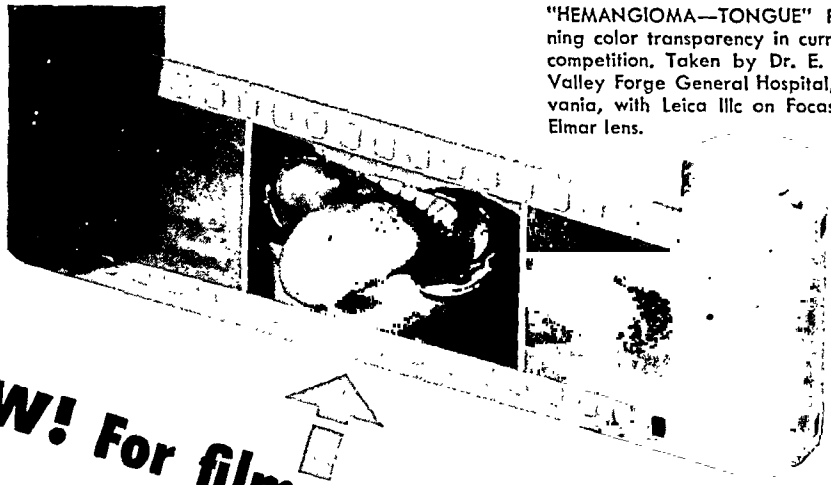
If you do not have copies on file of our General Laboratory Apparatus and Equipment Catalog No. 102 and Moduline Furniture Bulletin No. T-300, send for them today.

a. s. aloe company *Serving Science Since 1860*

1831 Olive Street • St. Louis 3, Missouri

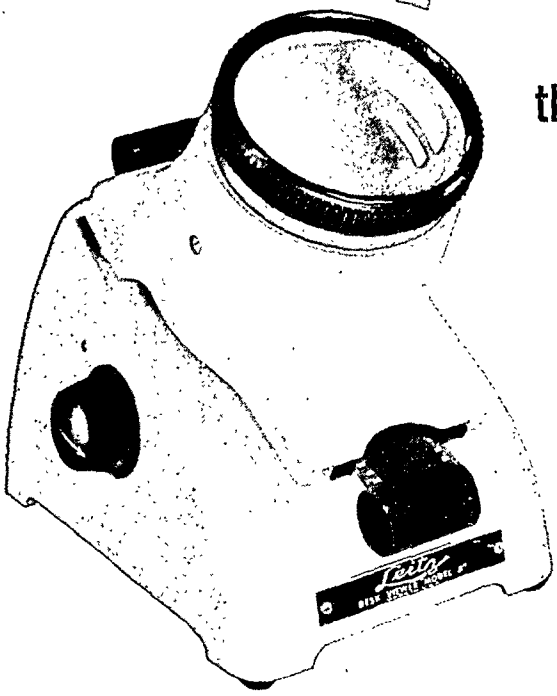
aloe

(In writing to advertisers, please mention the Journal—it helps.)



"HEMANGIOMA—TONGUE" Prize-winning color transparency in current Leica competition. Taken by Dr. E. K. Kelly, Valley Forge General Hospital, Pennsylvania, with Leica IIIc on Focaslide with Elmar lens.

NOW! For film strips and color slides



the Leitz

DESK VIEWER

The Leitz Desk Viewer shows your clinical photographs as they really are, bringing out with extreme sharpness all the detail and color gradation your camera actually caught. Magnified without distortion by a color-corrected optical system and a wide-field lens, the entire picture is as easily visible to both eyes as the page you are reading now.

The Desk Viewer accommodates both color slides and 35mm. film strips with rapid and smooth change-over. Its interchangeable roll film holder swings to *horizontal* or *vertical position,* a feature found in no other viewer.

Both mechanically and optically the Desk Viewer has the precision and craftsmanship associated with Leitz Microscopes and Leica* Cameras. Make a date today with your Leitz medical supply dealer or Leica Camera dealer to see the Desk Viewer . . . see how it brings out the best in your own clinical work.

VARIABLE MAGNIFICATION to suit individual preference by rotation of focusing mount . . . **EVEN ILLUMINATION** eliminates "hot spots" . . . **BUILT-IN RHEOSTAT** controls light intensity . . . **OPTICS**—a fine, wide-angle, triple-element achromatic lens . . . **ROLL FILM HOLDER** of exclusive Leitz construction permits easy viewing of both vertical and horizontal transparencies in the same strip, eliminates binding and scratching of film . . . **SLIDE TRACK** takes 3 glass slides (2" sq.) at a time.

PRICE—$66 . . . FILM HOLDER—$12 . . . CARRYING CASE—$5.

*Reg. U. S. Pat. Off. Exclusive trademark of

E. LEITZ, Inc.

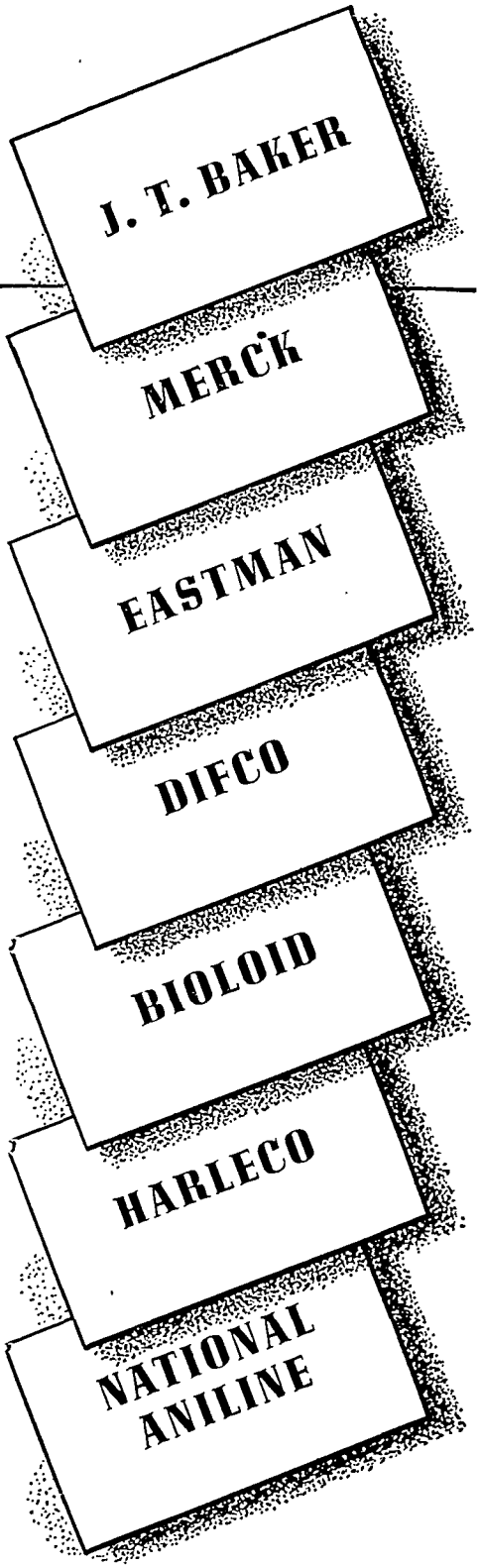
304 Hudson Street, New York 13, N. Y.

LEICA CAMERAS AND ACCESSORIES • LEITZ MICROSCOPES
BINOCULARS • SCIENTIFIC INSTRUMENTS

(In writing to advertisers, please mention the Journal—it helps.)

Names

that spell QUALITY in Laboratory Chemicals



You can rely on WILL and its affiliates for high quality laboratory reagents guaranteed by these outstanding names in the chemical industry.

WILL maintains four office-warehouses well stocked with inorganic and organic reagent chemicals, volumetric solution concentrates, pH indicators, culture media and a variety of bacteriological stains.

WILL offers well-known diagnostic reagents staining solutions, and reagents for blood and urine analysis.

WILL features its own "BIOLOID"* line of clearing fixing, mounting and embedding substances for microscopy.

For a convenient and single source of supply for all your laboratory chemicals, *write, teletypewrite, wire or phone our nearest office-warehouse listed below.*

BIOLOID

* BIOLOID TRADEMARK
(Reg. U.S. Pat. Off.)

SERVING SCIENCE THROUGH SUPPLY

WILL CORPORATION
LABORATORY APPARATUS AND CHEMICALS
ROCHESTER 3, N.Y. • NEW YORK 12, N.Y.
SOUTHERN SCIENTIFIC CO., INC., ATLANTA 3, GA.
BUFFALO APPARATUS CORP., BUFFALO 5, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)

we say they're better

and so will you when you've

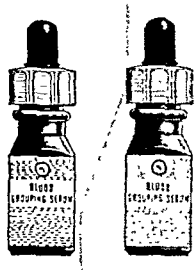
proved it in your own laboratory

HYLAND BLOOD GROUPING SERUM

Anti-A and Anti-B

Prepared from carefully selected donors to assure a highly potent, rapid acting, and specific product. Accurate results with maximum speed.

Available in 2 cc. and 5 cc. sets.



HYLAND ANTI-Rh SERUMS

Anti-Rh_0 (Anti-D) Typing Serum

Anti-Rh_0 rh' rh" (Anti-CDE) Typing Serum

Outstanding serum for slide method—rigidly tested for titer, avidity, specificity and sterility. Prepared from selected, immunized donors.

Both types available in 2 cc. and 5 cc. sizes.



make this test at our expense!

Clip the coupon for your free samples today! You'll receive generous samples of Rh_0 Typing Serum and Blood Grouping Serum. Enough to convince you that their speed, accuracy and titer are unsurpassed.

Hyland Laboratories

4534 Sunset Boulevard, Los Angeles 27, California

Please send me generous samples of Rh_0 and Blood Grouping Serums.

Your Name_____

Hospital or Lab. Name_____

Address_____

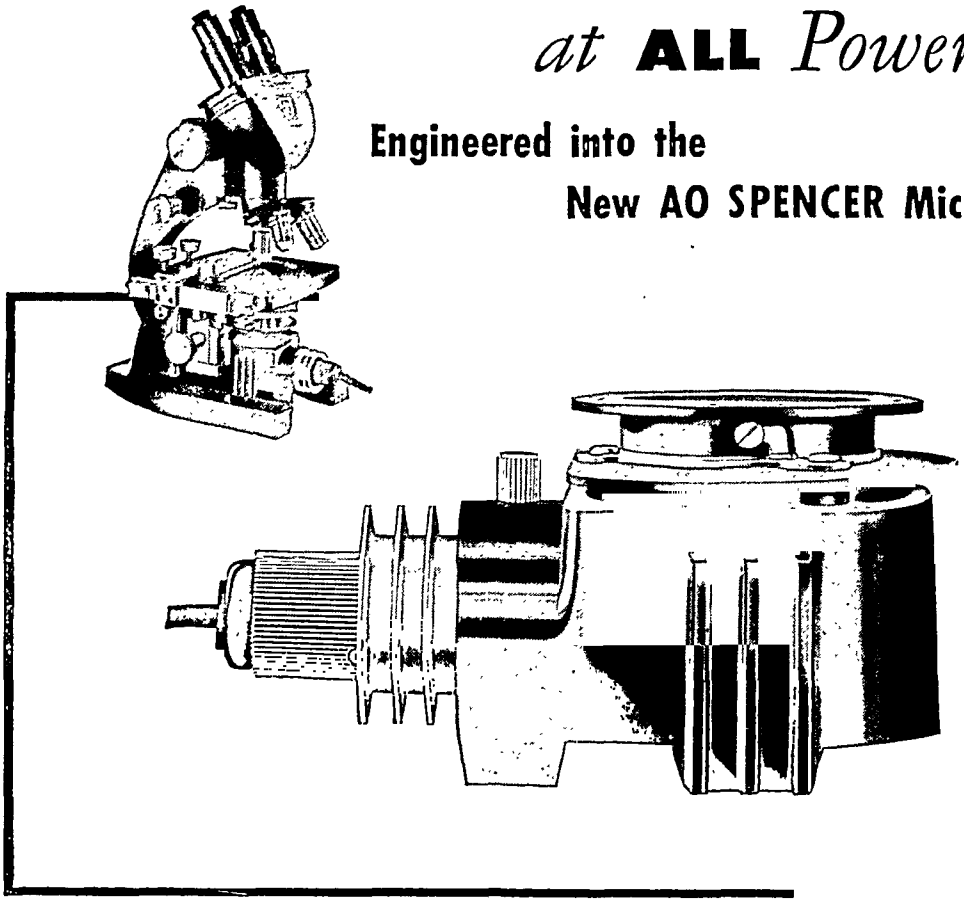
City and State_____

From what dealer do you regularly buy your typing serums?_____

(In writing to advertisers, please mention the Journal—it helps.)

FULL FIELD *Illumination at* ALL *Powers*

Engineered into the New AO SPENCER Microscopes



FROM the lowest power to the oil immersion the new No. 700 Illuminator provides convenient, uniform illumination that adequately fills the field without refocusing the condenser. This light source has been engineered to function as an integral part of the new AO Spencer Microscopes—yet it can be removed or attached at a moment's notice. With the illuminator in position the microscope can be inclined at any angle or moved about the table without need for realigning the illuminating system. *Cooling fins, heat-absorbing glass,* and baffle plate all help maintain a constant temperature suitable for living specimens. Other features are: *bulb with built-in reflector, easy access to bulb, switch-in cord.*



For your copy of a catalog illustrating the complete new line of AO Spencer Laboratory Microscopes, the No. 700 Illuminator, and other accessories, write Dept. X16.

American Optical Company
Scientific Instrument Division
Buffalo 15, New York

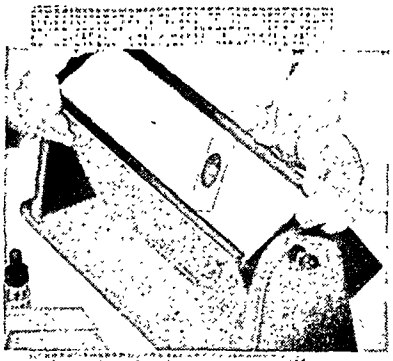
Makers of Microscopes for over 100 Years

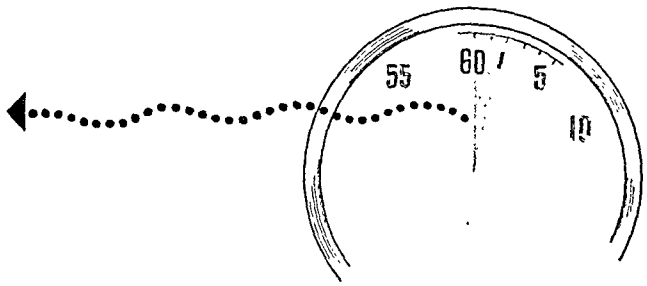
SPENCER

TWO drops of blood

ONE drop of anti-(Rh)serum

. . . Test complete in TWO minutes or less

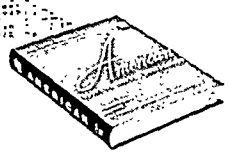




Rapid, accurate, satisfying results in blood testing are a certainty with Rh serums from AMERICAN. No microscope is needed. The Slide Method gives clear, distinct, unmistakable readings to the naked eye. Clumping starts in 45 seconds—is complete in from 1 to 2 minutes.

These potent and stable serums may be used with fresh, clotted, oxalated, heparinized or citrated blood. Serums are prepared from immunized *human* donors in a Government licensed laboratory—in strict accord with NATIONAL INSTITUTE OF HEALTH specifications.

WRITE TODAY *for descriptive literature on* AMERICAN'S *complete line of blood-typing and blood-grouping serums.*



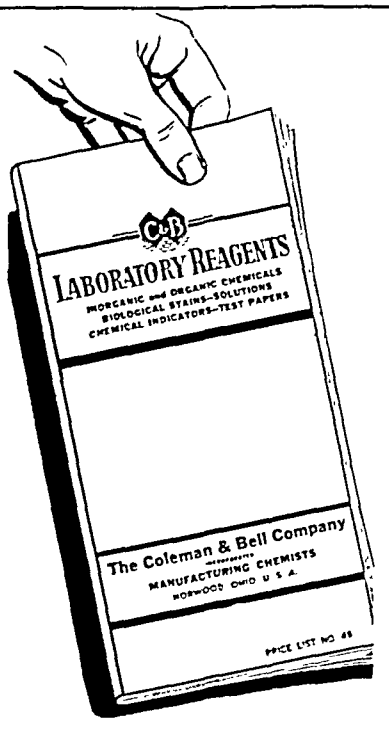
PLAN WITH AMERICAN
. . . the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

C&B

LABORATORY REAGENTS



New Catalog
FREE

If you have not received a copy of this new catalog please notify us at once.

Inorganic and
Organic Chemicals

•

Biological Stains
Solutions

•

Chemical Indicators
Test Papers

•

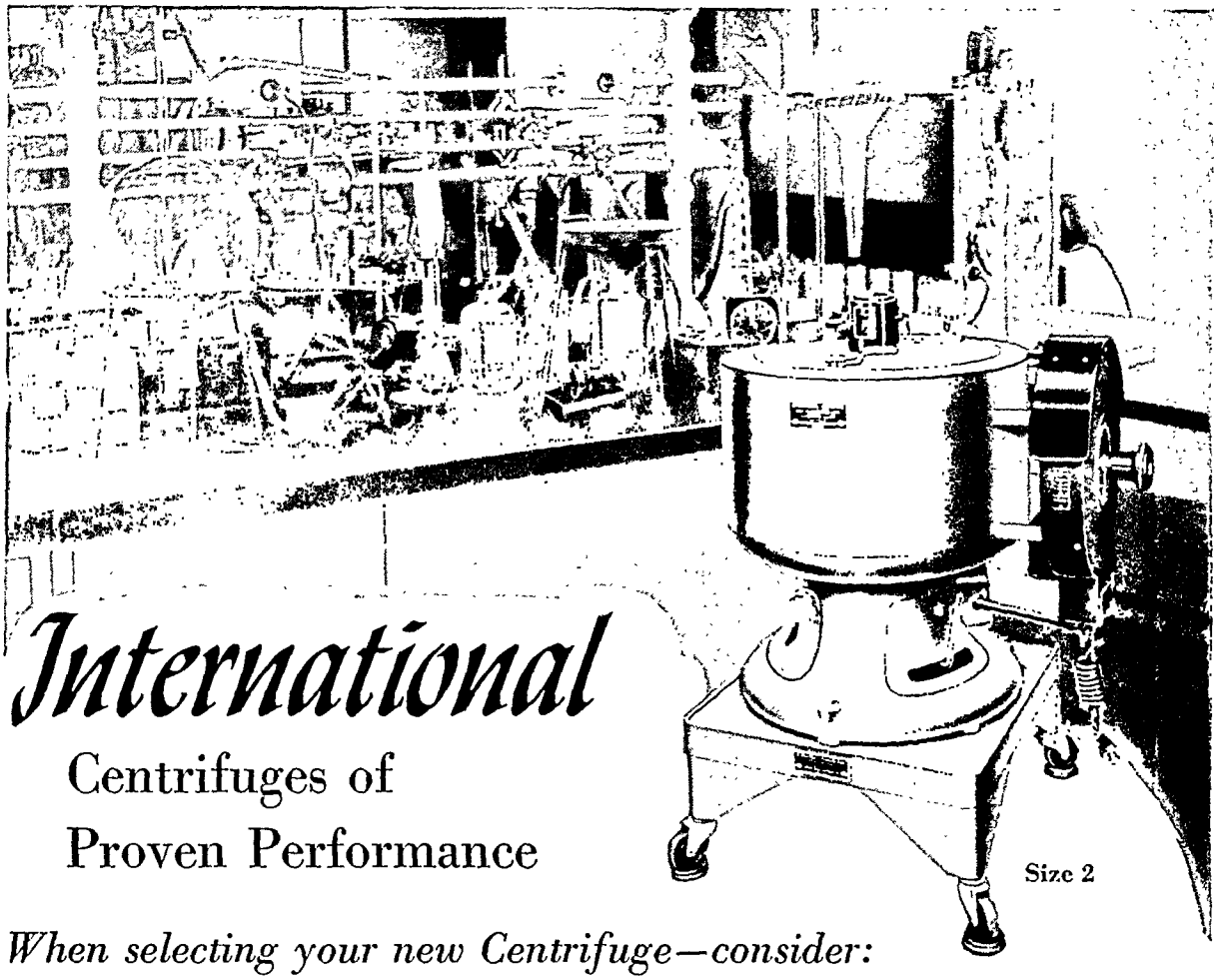
Distributed by
Physician and Laboratory Supply Houses

The COLEMAN & BELL Co., Inc.

Manufacturing Chemists : Norwood, Ohio, U. S. A.

COLEMAN & BELL

(In writing to advertisers, please mention the Journal—it helps.)

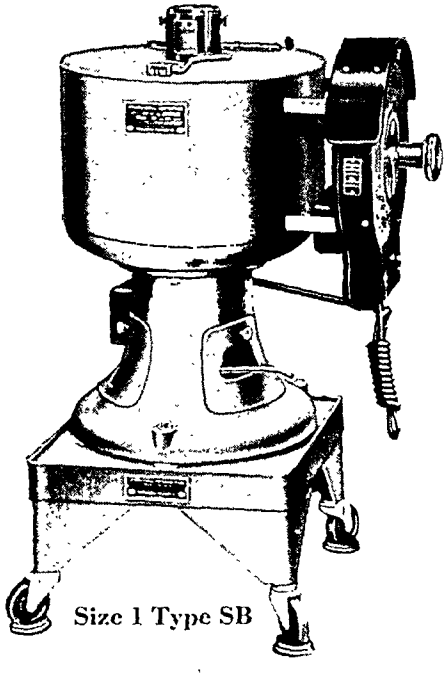


International

Centrifuges of Proven Performance

Size 2

When selecting your new Centrifuge—consider:



Size 1 Type SB

1 **The reliability of the electric motor that drives it.**
The motors of International Size 1 and Size 2 Centrifuges are specially designed to provide the best possible drive for a Laboratory Centrifuge. They are manufactured entirely in our own shops, thus eliminating the possibility of obsolescence through discontinuance by an "outside" manufacturer.

2 **The range of its application.**
Interchangeable accessories designed specifically for International Centrifuges are available in wide variety, making the machines adaptable to practically every laboratory application. Your future requirements as well as your present needs should be considered.

3 **The relation of first cost to total cost.**
Expensive repairs can make a low first cost a high cost in the long run. International Centrifuges are famous for their ability to give years of trouble-free service.

4 **The reputation of the manufacturer.**
International's fifty years of specialization in the manufacture of Laboratory Centrifuges is your guarantee of unequalled performance.

INTERNATIONAL EQUIPMENT COMPANY

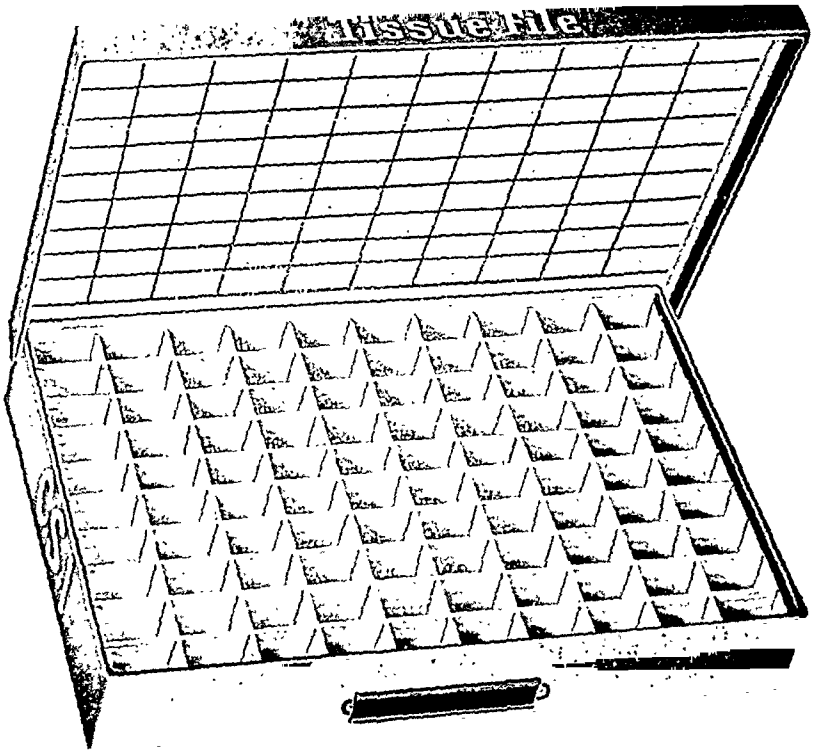
1284 SOLDIERS FIELD ROAD, BOSTON 35, MASS.

The Cargille

TISSUE FILE

For filing and storing Histology Tissues Embedded in Paraffin or Celloidin Blocks.

Will also prove indispensable in anatomy and other biological laboratories where tissues are studied by histological methods.



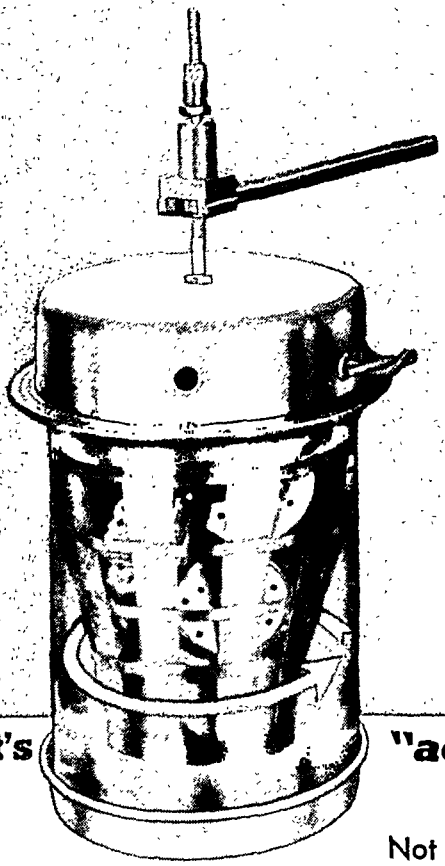
- Conveniently stores one hundred groups of blocks in one case. Each compartment will accommodate 10 to 15 blocks of the usual size.
- Removable partitions make it possible to double or quadruple the size of compartments.
- Indexing data (date, number, etc.) can be recorded on card in front of case.
- Inside of hinged cover is ruled into spaces corresponding to compartments. Data for indexing each lot of specimens can be recorded in proper space for instant reference.
- Sturdily constructed, specially reinforced. Designed so that each case can be conveniently numbered and the cases stacked on shelves.

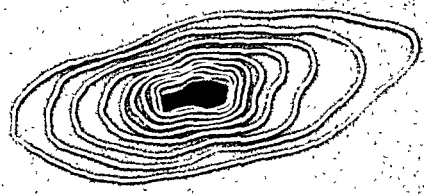
● Case measures $15\frac{1}{4}'' \times 10\frac{1}{2}'' \times 2\frac{3}{4}''$ and is divided into 100 compartments, each measuring $1\frac{1}{2}'' \times 1'' \times 2\frac{1}{4}''$ deep. Shipping weight for each case $3\frac{1}{2}$ lbs. PRICE, 1 to 11 . . $2.75 ea; 1 doz. $27.50

CARGILLE SCIENTIFIC INC.

118 LIBERTY STREET NEW YORK 6, N. Y.

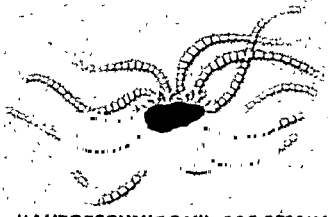
(In writing to advertisers, please mention the Journal—it helps.)





"HAND" PROCESSING

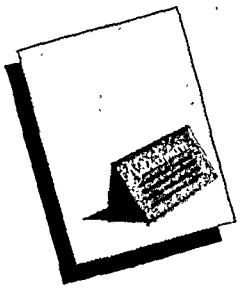
The tissue, lying at rest merely steeping in a reagent, is surrounded by a "skin" of relatively inactive solution. Permeation is slow, uncertain.



"AUTOTECHNICON" PROCESSING

The tissue moves about in the fluid, as it revolves in the carrier basket. Fresh surfaces are constantly being presented for penetration by a reagent which is, itself, in a state of gentle agitation through the constant rotation of the basket.

it's *"active displacement"* that does it



A brochure describing the Autotechnicon is available on request.

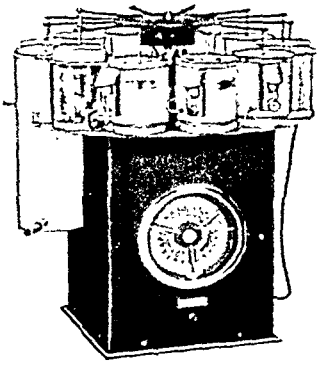
Not only does the **Autotechnicon** relieve the laboratory staff of the tedious task of processing tissues "by hand," but it also completes the chosen technique *faster, more thoroughly, more uniformly, more dependably.* The **Autotechnicon** is versatile . . . it fixes, dehydrates, washes, stains . . . delivers beautifully finished tissues ready for diagnosis.

And because it needs no human intervention, it knows no "working day." To-day's tissues, processed overnight by your own technique, will be waiting for you in the morning.

the Autotechnicon

Trade Mark Registered U S Patent Off

automatic tissue processing by any histologic technique



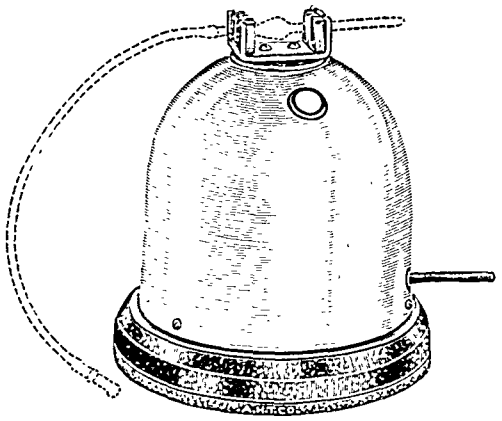
THE TECHNICON COMPANY

215 EAST 149th STREET • NEW YORK 51, N.Y.

A.H.T. CO. SPECIFICATION

BLOOD PIPETTE SHAKER

With automatic shaking control and shutoff, providing quick, effective and reproducible shaking



3426-A.

SHAKER FOR HEMACYTOMETER PIPETTES, Research Model, Electric, A.H.T. Co. Specification. For quick and effective shaking of diluting pipettes in a reproducible manner. The automatic shaking action, requiring approximately thirty seconds, comprises 1200 rapid, transverse vibrations of small amplitude, with automatic shutoff, reducing the possibility of hemolysis due to mechanical rupture and fragmentation of fragile erythrocytes.

Consisting of a shaded pole electric motor fitted with eccentric drive and spring device for converting continuous into vibratory motion; gear train with cam and micro switch for limiting the predetermined number of vibrations; all enclosed in dome-shaped aluminum housing on cast metal base, with push button for starting, and aluminum pipette holder with nickel-plated spring bronze clips.

The pipette holder is a simple device which permits insertion or removal of pipette with one motion. The type and amplitude of the stroke make it possible to shake the pipette efficiently with both ends open but, if preferred, shaking can be accomplished without removing rubber tubing, mouthpiece or closure. Overall dimensions 6½ inches diameter x 8 inches high; current consumption 0.1 ampere.

CAUTION. Blood cells begin to settle immediately after vibration ceases and delay in filling the counting chamber, particularly for red cell count, will introduce appreciable error. While this Shaker is offered with a two-place head, *we do not recommend shaking more than one pipette at a time* except when making routine counts of red and white cells on the same specimen, or when used simultaneously by more than one technician.

3426-A. Shaker for Hemacytometer Pipettes, Automatic, Research Model, Electric, A.H.T. Co. Specification, as above described, with two-place head, thick sponge rubber pad, 5-ft. connecting cord, switch and plug. For use on 115 volts, 60 cycles, single phase, a.c. only..... 40.00
10% discount in lots of 6 or more.

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

CABLE ADDRESS, "BALANCE", PHILADELPHIA

(In writing to advertisers, please mention the Journal—it helps.)

I. EXPERIMENTAL CORTICAL NECROSIS OF ADRENAL GLAND IN THE MONKEY IN DIPHTHERIA AND IN MALARIA

With Observations on a Fatal Human Infection with *Plasmodium falciparum**

ALFRED GOLDEN, M.D.,† and RICHARD R. OVERMAN, Ph.D.

From the Pathological Institute and the Department of Physiology, University of Tennessee College of Medicine, Memphis, Tennessee

Epidemic typhus fever as seen in Guatemala in 1944, in addition to the usual skin eruption and fever, was characterized clinically by extreme prostration, blood pressure readings at shock levels and collapse of the superficial veins. In ten subjects that came to autopsy, our attention was drawn particularly to necrotic foci in the adrenal cortex, which was reported.[1] Identical lesions have been reported by Rich[5] and by Kinsman *et al.*[3] in a variety of infectious diseases including meningococcic septicemia.

The present report is concerned with the experimental production of such lesions in monkeys (*Macacus mulata*) and with observations in a fatal human infection with *Plasmodium falciparum*. Biochemical determinations including blood sodium and potassium and total blood volume in the monkeys, are being reported in a separate communication.[4]

METHOD

The lesions to be described may be confused by some with postmortem autolytic changes. To avoid any such confusion, moribund animals were sacrificed by deep pentothal anesthesia and the adrenal glands were removed by laparotomy as soon as surgical anesthesia was induced. The findings in two animals that were autopsied will be noted below. The glands were fixed immediately in either neutral 10 per cent formaldehyde solution or in Zenker's fluid. Paraffin-imbedded sections were stained by hematoxylin and eosin, iron-hematoxylin, azocarmine, Wilder's reticulum and Giemsa's methods. Each adrenal gland was cut into 6 to 10 blocks and from each block every tenth section was stained and kept for study.

A freshly isolated human strain of *Corynebacterium diphtheriae* was inoculated into the deep lumbar muscles of two monkeys to determine the changes produced in the adrenal cortex. This was done within two days after it had been demonstrated that 2 ml. of a broth culture of this organism had produced death in two guinea pigs in 27 hours, with typical hemorrhagic necrosis of the adrenal glands.

Observations were then extended to experimental infection in monkeys with

* Presented, in part, before The Society for Experimental Pathology, 1947.
This work was supported in part by a grant from the U. S. Public Health Service.
Received for publication, June 6, 1949.

† Present address: Veterans Administration Hospital, Batavia, New York.

a strain of *Plasmodium knowlesi*.* After intravenous inoculation of blood from a chronically infected monkey, the acute simian disease is characterized by progressively increasing parasitemia, fever, severe progressive anemia and prostration, and decrease in the hematocrit reading to 50 per cent or less of normal. The duration of the experimentally induced disease varied from 4 to 12 days. Maximum degrees of parasitemia, usually in the terminal stage of the disease, ranged from 9.2 per cent to 90.2 per cent (thin-film counts). In this series, 6 monkeys were observed until they were moribund.

We then decided to observe the effect of adrenal cortical hormone on the microscopic appearance of lesions of the adrenal glands. Lipo-adrenal extract† was given intravenously to *one* of a pair of simultaneously infected monkeys. Five such pairs of animals, 10 in all, were studied with no other medication or hormonal substitution-therapy, in an experiment designed not to test the therapeutic efficacy of adrenal cortical hormone therapy, but only to determine the effect on the necrotic lesions (to be described below) and on the biochemical changes. Each of 3 monkeys received 5 ml. of the extract intramuscularly on the day the thick film first showed organisms, and the same dose once daily until the animal was sacrificed. Two monkeys received the same dose simultaneously with the inoculation of plasmodia. Total quantities ranged from 20 ml. in each of 3 animals to 50 ml. in one animal.

Control observations were made on the adrenal glands of two monkeys. One of these animals died accidentally from hemorrhage following venipunctures. The other was sacrificed because of a strongly positive tuberculin skin reaction and, at autopsy, tiny granulomatous lesions were found along the dorsal surfaces of one lung.

During the course of the experiment, a 19 year old white woman died in the hospital from an infection with *Plasmodium falciparum*. Her adrenal glands were removed at autopsy less than one hour after death.

RESULTS

The guniea pigs that died of *C. diphtheriae* infection grossly showed massive hemorrhage into the adrenal glands. Microscopically, adjacent to such hemorrhages were small foci or large patches of either coagulation necrosis or simple solution of cells chiefly in the zona fasciculata, and occasional areas of necrosis were also present in the zona glomerulosa and zona reticularis. There was no leukocytic reaction to either the hemorrhages or focal necroses.

The 2 monkeys that were inoculated with diphtheria organisms responded differently. One animal was moribund in 48 hours and was sacrificed. At laparotomy the adrenal glands grossly were not hemorrhagic. Microscopically, there were extensive zones of bland necrosis involving few cells, occasionally associated with hemorrhages apparently derived from the adjacent sinusoids. The

* Secured from Dr. Quentin Geiman of Harvard University, Boston, Massachusetts.

† Supplied through the courtesy of Dr. H. F. Hailman of the Medical Division of the Upjohn Co., Kalamazoo, Mich. This extract contains no desoxycorticone acetate.

loss of cell substance appeared to be by solution. As a result of this toxic form of necrosis, glandlike spaces appeared in what was once a solid cord of cells (Fig. 1). Reticulum stains showed loss of staining of the fibers, presumably because of destruction, chiefly in the areas of hemorrhage. The other monkey, a larger and heavier animal, was sacrificed after 96 hours, even though symptomless. The adrenal glands showed microscopically only one area of hemorrhagic necrosis but several small areas of bland necrosis, like those described above, all devoid of leukocytic reaction. The only patch of destroyed reticulum was in the area of hemorrhagic necrosis.

The two control animals showed histologically normal adrenal glands. Vacuolization, interpreted as lipoid accumulation, was variable even in the same section but was never marked. The continuity of the cortical cords of cells was uninterrupted throughout. No lesions resembling in any way those to be described were encountered in the large number of sections examined. No lesions were seen in the medulla of the adrenal gland in experimental or in control animals.

The gross and microscopic findings at autopsy of the human malarial infection, as well as of the experimental simian malarial infections, were entirely typical of overwhelming infections that are fatal in the first attack. The spleens were firm and reddish black, the livers had a brownish black cast, were swollen, and there was evidence of anemia and dehydration. Microscopically, malarial pigment was abundant in the liver, spleen, lymph nodes and bone marrow. Vessels throughout the body contained parasitized erythrocytes. There was no evidence of chronic malarial infection, such as fibrosis of the splenic capsule or pulp in any of these cases.

All of the untreated animals experimentally infected with *P. knowlesi* showed lesions in the adrenal cortex, distributed most plentifully in the zona fasciculata, but not sparing entirely the zona glomerulosa or zona reticularis. Only minute foci of hemorrhage were encountered. There was no recognizable destruction of reticulum or collagen. Prominent granularity and/or vacuolization was also noted in these cells with great frequency.

The iron-hematoxylin and azocarmine stains demonstrated best the following changes. The earliest recognizable change was a loss of the cell border and, where adjacent cells were affected, there was a mingling of their cytoplasm (Figs. 2–5). There was greater loss of cytoplasm and apparently of nuclei when the lesion was more pronounced, leaving no trace of cell substance. As a result there was a defect in the continuity of the adrenal gland cords which were so frequently oval or circular as to resemble tubule formation. No stainable substance, other than bits of cellular debris, was present in these lakes or pseudo-tubules which were bordered by histologically-intact cells or others showing beginning solution. Pyknosis of nuclei was common. Occasionally, small numbers of intact erythrocytes were seen in these defects, but never any leukocytic reaction. In the most severe form of this lesion, every microscopic field showed some change. The severity of the lesions was judged microscopically by the number of cells involved.

Without exception, the administration of the lipoidal adrenal cortical ex-

tract in the doses given, during this malarial infection, either prevented the appearance of the lesion entirely or reduced its severity so that only minute foci of 3 to 5 necrotic cells could be found in occasional sections.

Animals treated with lipoidal adrenal cortical extract lived up to 12 hours longer than untreated animals (an increase of from 5 to 12 per cent in time of survival). This is considered as being biologically (but not statistically) significant, particularly because no attempt was made to treat the parasitemic level, the anemia, fever, or any of the other serious aspects of this infection.

In the fatal human infection with *P. falciparum*, the adrenal cortex showed the most pronounced lesions seen in this series. Histologically, normal cells were in the minority, and small areas of hemorrhage frequently obscured the "tubule formation" resulting from the necrosis of cells. In cases where whole cords were destroyed, collapse of the sinusoids was demonstrated well by the azocarmine stains. Pyknosis of nuclei was common in either partially preserved cells or in those showing only a ragged remnant of cytoplasm (Figs. 6-8). In many places polymorphonuclear leukocytic reaction was prominent but not necessarily associated with focal hemorrhages (Fig. 7).

DISCUSSION

It is well known that the adrenal glands may undergo such severe autolytic change, even early postmortem, as to make histologic interpretation of any kind difficult. The changes described are distinct from those of simple autolysis. Similar necrotic lesions could not be reproduced by intentional exposure of human adrenal glands to room temperature for as long as 10 hours, or by similar treatment of a slice of adrenal gland of one of the normal monkey controls. It must be understood that autolytic changes may obscure the necrotic lesions, particularly in their early stages. In this respect the lesions are not unlike those seen in the kidney in acute tubular necrosis (in lower nephron syndrome, hemoglobinuric nephrosis, or "crush" kidney), where the changes vary from excessive cytoplasmic granularity and/or vacuolization to frank necrosis. In order to avoid

FIG. 1. Lacuna or tubule formation in experimental monkey diphtheria, in the zona fasciculata of the adrenal gland. Early changes are seen at A and are characterized by solution of cells, leaving defects in the continuity of cell cords. Pyknotic nuclei are seen at B and focal hemorrhages into the defects in the cords at C. Iron-hematoxylin. × 450.

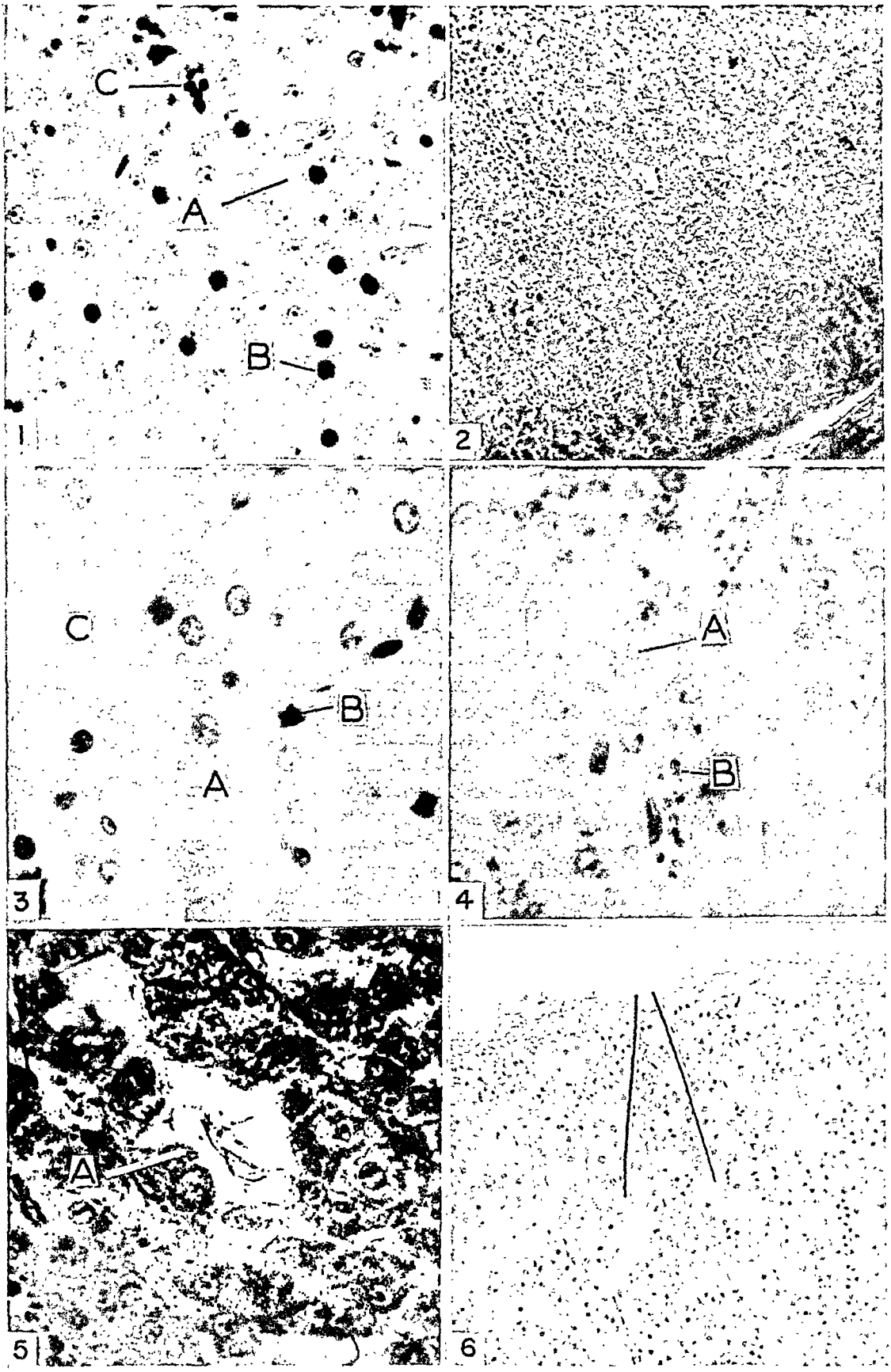
FIG. 2. Adrenal cortex in experimental monkey malaria. The zona glomerulosa stains deeply with iron-hematoxylin in contrast to the zona fasciculata, which contains numerous lacunae. Iron-hematoxylin. × 120.

FIG. 3. High power view of one of the lesions seen in Figure 2. Note loss of cell borders at A, early pyknosis of nuclei at B, and formation of defects in the continuity of the cortical cell cords at C. Hematoxylin and eosin. × 450.

FIG. 4. High power view of cellular changes in adrenal cortex in monkey malaria. Two erythrocytes (A) can be seen passing from a regional sinusoid into a zone of early necrosis. Parasitized erythrocytes within sinusoids are seen at B. Azocarmine stain. × 350.

FIG. 5. In this zone of necrosis within the adrenal cortex, note that the defect is bordered by cells with histologically-intact cell borders (A). Compare with Figure 3. The material within the newly-formed lumen is consistent with cytoplasmic debris. The marked granularity of the cell cytoplasm in the cells bordering the defect is well brought out by this stain. Iron-hematoxylin. × 450.

FIG. 6. Zona fasciculata of adrenal cortex from fatal human infection with *P. falciparum*. The ragged defects (A) in the cords of the cortex are identical to those seen in the experimental animals. Hematoxylin and eosin. × 120.



FIGS. 1–6

complicating postmortem change, all but two experimental animals were subjected to laparotomy under pentothal anesthesia and their adrenal glands were removed before death and fixed immediately. Of the two animals that were not so sacrificed one was autopsied within 30 minutes after death, and the other within 90 minutes In neither of these animals did the adrenal glands show appreciable autolysis microscopically, nor were the findings in these animals different qualitatively from any of the others. In the case of the woman with fatal malaria, the adrenal glands were removed within one hour after death and placed in formalin fixative. No appreciable autolytic changes were seen microscopically in many sections taken from the adrenal glands.

The correlation between the severity of the adrenal cortical lesions and the degree of biochemical change was fairly close. Those animals showing the greatest percentage shifts of sodium into erythrocytes (increases up to 204 per cent) showed the largest and most widespread necrotic foci in the adrenal glands. On the other hand, reversal or prevention of the adrenal necrosis by lipoid cortical extract was not reflected entirely in a reversal of the biochemical alterations. The details of these findings will be reported separately.

The adrenal cortical lesions are for the most part devoid of any kind of inflammatory response. In the fatal human infection of malaria there was polymorphonuclear leukocytic reaction in the necrotic foci. It seems reasonable to assume that this inflammatory response is a late phenomenon which was probably interrupted by sacrificing the moribund animals in this experiment. The products of cellular necrosis by solution appears to stimulate but little inflammation. In this respect there is a parallel in the necrosis in renal tubules and in the liver as well with the early stages of coagulative necrosis of liver cells in yellow fever, and in certain stages of acute hepatic necrosis produced by a variety of causes.

A close correlation exists between the severity of the adrenal gland necrosis and of the experimental malarial infection, as demonstrated by the degree of parasitemia and by the shift of the sodium-potassium balance, particularly of sodium into the erythrocyte. That a biochemical reversal could not be demonstrated along with an obliteration or reversal of the adrenal cortical lesion on substitution therapy alone is not to be taken as an indication of the failure of such therapy. It was anticipated before the experiment that the administration of adrenal cortical hormone would show an effect chiefly on the sodium and potassium imbalance, but we did not anticipate visible changes in the gland itself. The possible mechanisms of prevention of the adrenal lesion or the protection of vulnerable gland cells by this hormone are not obvious from this experiment. The therapeutic implications of this procedure must await further investigation.

SUMMARY

Necrosis of the adrenal cortex occurs regularly in experimental simian malaria (*Plasmodium knowlesi*). The severity of the lesion is roughly proportional to the severity of the infection and is reflected biochemically most accurately in the degree of shift of sodium into erythrocytes. The lesions can be prevented entirely or there can be a reversal of these lesions by the administration of lipoidal ad-

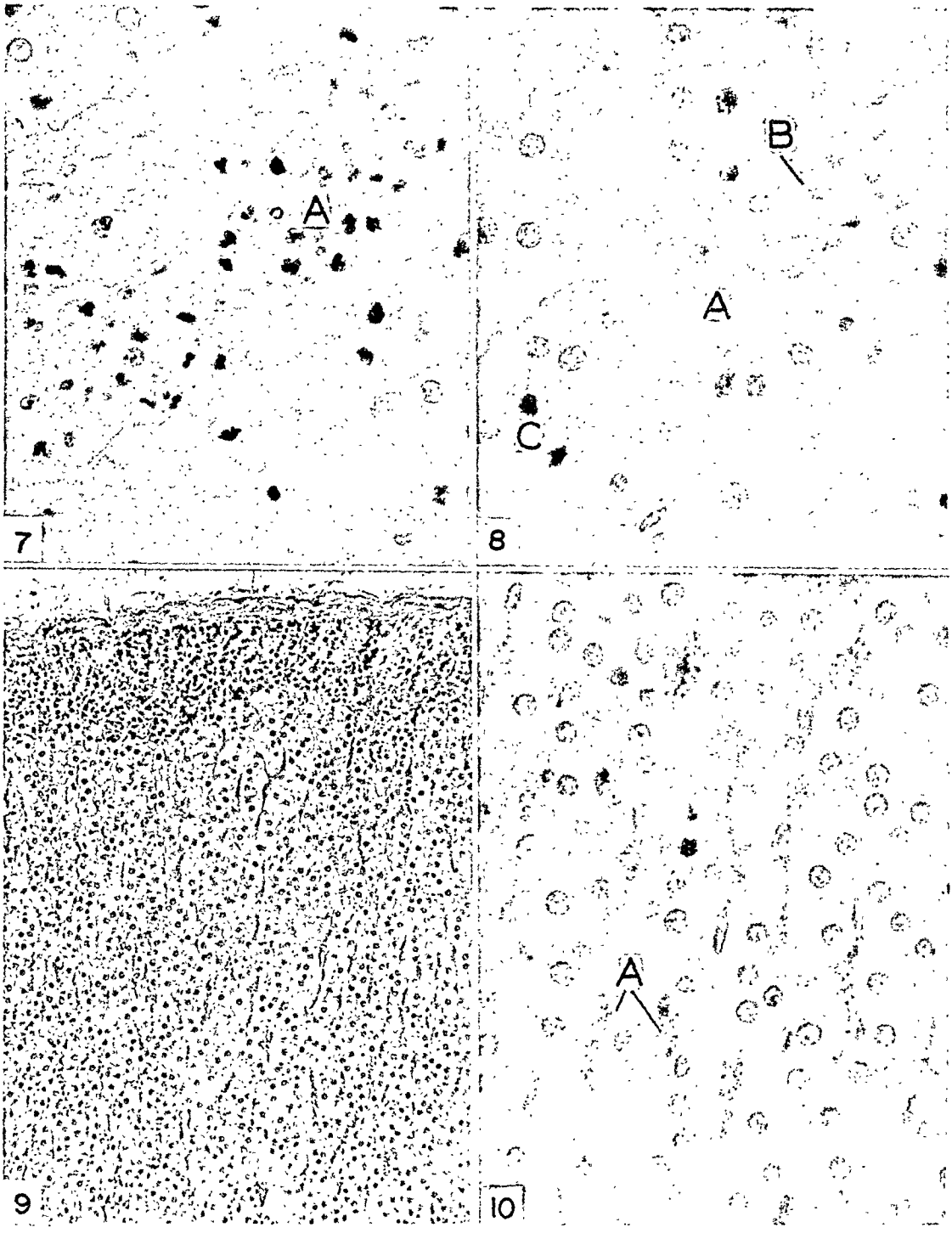


FIG. 7. From case of human malaria. In many areas of the adrenal glands clusters of polynuclear leukocytes (A) infiltrate into the zones of necrotic cells. Hematoxylin and eosin. × 450.

FIG. 8. From same case of human malaria. Note pseudotubules or lacunae at A, ragged cytoplasm at B, and pyknotic nuclei at C. Hematoxylin and eosin. × 450.

FIG. 9. Cortex of adrenal gland showing zona fasciculata and zona glomerulosa, from a monkey that received lipoidal adrenal gland extract on the day the thick blood-film first showed organisms. A total of 5 doses of the extract was given intramuscularly to this animal. The histologic picture is indistinguishable from that of the normal control animals and is identical to that of all monkeys receiving this hormone. Hematoxylin and eosin. × 120.

FIG. 10. From the same animal showing in detail the parasitized erythrocytes in the adrenal gland sinusoids (A), and the well preserved adrenal gland cells with their sharply defined cell borders. The cytologic picture is indistinguishable from that of normal control animals. Hematoxylin and eosin. × 450.

renal cortical extract in the presence of otherwise untreated malarial infection. The lesions are identical qualitatively to those that can be produced experimentally by intramuscular inoculation of *Corynebacterium diphtheriae* into guinea pigs and monkeys. This is considered significant inasmuch as one of the accepted sites of action of diphtheria toxin is in the adrenal cortex. An identical lesion, but of much greater severity, was seen in a fatal human infection with *P. falciparum*.

CONCLUSIONS

1. Focal adrenal cortical necrosis, such as is seen in epidemic typhus, can be produced experimentally in guinea pigs and monkeys by inoculation of virulent diphtheria organisms, and in monkeys by malarial infection with *Plasmodium knowlesi*.

2. Identical but more severe lesions were found in the adrenal glands of a woman with an overwhelming infection with *Plasmodium falciparum*.

3. The adrenal lesions in monkeys dying of experimental malaria can be prevented by administration of lipoidal adrenal cortical extract.

REFERENCES

1. GOLDEN, A.: Morbid anatomy of typhus as seen in a recent Guatemalan epidemic; observations on the disease. Arch. Path., **39**: 226–231, 1945.
2. GOLDEN, A., AND OVERMAN, R. R.: Sodium-potassium levels and adrenal gland necrosis in experimental simian malaria. Federation Proc., **7**: 270–271, 1948.
3. KINSMAN, J., D'ALONZO, A. C., AND RUSSI, S.: Fulminating meningococcic septicemia associated with adrenal lesions; analysis and discussion of seven cases. Arch. Int. Med., **78**: 139–169, 1946.
4. OVERMAN, R. R., BASS, N. E., DAVIS, A. K., AND GOLDEN, A.: The effect of lipo-adrenal extract on ionic balance in fatal simian malaria. Am. J. Clin. Path., **19**:907–917, 1949.
5. RICH, A. R.: A peculiar type of adrenal cortical damage associated with acute infections, and its possible relation to circulatory collapse. Bull. Johns Hopkins Hosp., **74**: 1–15, 1944.

II. THE EFFECT OF LIPO-ADRENAL EXTRACT ON IONIC BALANCE IN FATAL SIMIAN MALARIA*

R. R. OVERMAN, PH.D., ANNE C. BASS, A. B., A. K. DAVIS, B. S., AND ALFRED GOLDEN, M.D.†

From the Department of Physiology and the Pathological Institute, University of Tennessee College of Medicine, Memphis, Tennessee

It has been previously reported from this laboratory[13] that the plasma levels of sodium, potassium, and chloride are almost unaltered during the course of fatal infections with *Plasmodium knowlesi* in monkeys. However, relatively enormous changes in the concentrations of these ions are found in nonparasitized erythrocytes of the infected animals. It has been shown, for example, that the average red blood cell sodium concentration is increased 100 per cent before death with a concomitant reduction in erythrocytic potassium and a rise in chloride levels. Further, the urinary excretion of Na and Cl have been shown to decrease to low levels, while that of K rises until late in the course of the malaria when the animals become oliguric or anuric. These alterations in distribution and renal excretion of ions are reversible when chemotherapeutic intervention is made early in the course of the disease.[10, 11, 12, 13, 17]

These profound alterations in native ionic balance had been previously predicted[10, 16] from data regarding the distribution of thiocyanate in simian *P. knowlesi* and human *P. vivax* and *P. falciparum* infections. The *in vivo* demonstration of severe electrolyte imbalance together with numerous necropsy reports[2, 3, 8, 9, 18] of adrenal cortical necrosis in cases of fulminating malarial infections in human beings and monkeys led to the present investigation. Since the physiologic integrity of the adrenal cortex is known to be necessary for normal distribution of Na and K, we decided to determine the effects of adrenal extract on these chemical substances and on the course of malaria. We have previously confirmed the presence of adrenal cortical necrosis in untreated monkeys dying of *P. knowlesi* infections.[4, 5, 6]

MATERIALS AND METHODS

Control determinations were made of Na, K, and Cl concentrations of plasma and erythrocytes and the hematocrit values of 23 normal *Macacus mulata* monkeys. Each animal was subsequently infected with *P. knowlesi* by blood inoculation from an animal bearing a chronic infection. A total of 77 serial determinations of these ionic levels were subsequently made at varying intervals until death of the animals.

* Supported by a grant-in-aid from the Division of Research Grants and Fellowships of the U. S. Public Health Service.

A portion of this work was presented before The Society for Experimental Pathology in March 1948.

Received for publication, June 6, 1949.

† Present address: Veterans Administration Hospital, Batavia, New York.

Eight of the 23 animals were each given 5 ml. of lipo-adrenal extract* daily by intramuscular injection. Sodium and potassium concentrations in plasma and packed erythrocytes were measured by internal standard flame photometry by the methods of Davis and Overman.[1, 14] Chlorides were determined by a modification of the method of Volhard.[19] Erythrocyte ionic levels were measured in nonparasitized cells obtained by centrifugation for one hour at 2300 G. Hematocrit readings were obtained by the Wintrobe tube method. Plasma protein concentration was determined by the copper sulfate method of Phillips *et al.*[20]

Blood samples for analysis were withdrawn by needle puncture from the femoral artery into oiled, heparinized† syringes and delivered under oil into collection tubes to prevent the "chloride shift". The femoral artery was not exposed for sampling and hematomas were prevented by stasis produced by the finger and by massage.

RESULTS

The *qualitative* ionic alterations in both untreated and adrenal extract-treated animals are similar to those previously reported by Overman.[13] However, when the progressive changes in the two series are plotted against time, a number of striking differences appear between the two groups. Since alterations occur in hematocrit values and in parasitemia at somewhat different rates in treated and untreated animals, we have logically but arbitrarily selected hours after inoculation with *P. knowlesi* as being the least equivocal of several possible bases for comparison.

Arbitrary groups of treated and untreated animals were established as follows, to contain approximately the same numbers of determinations: Group I, normal animals; Groups II, III and IV, malarious animals (untreated) measured at averages of 124, 160 and 183 hours, respectively, after inoculation with *P. knowlesi* (see Table 1). Animals comprising the adrenal extract-treated groups were divided and designated as follows: Group A, normal animals subsequently treated with 5 cc. doses of lipo-adrenal extract daily throughout the malarious course; Groups B, C and D, treated malarious animals measured at averages of 126, 182, and 199 hours after inoculation (see Table 2).

Length of life. In each case, the animal receiving daily injections of lipo-adrenal extract outlived its identically infected untreated control. All animals eventually died of malaria and the average prolongation of life in treated animals is not *statistically* significant. However, the prolongation of life appears to be of biologic significance when the differences to be reported subsequently are considered.

Hematocrit values. The average hematocrit reduction in untreated animals during

* We are indebted to the Upjohn Company, Kalamazoo, Michigan for generous supplies of this material. Lipo-adrenal extract is a pork adrenal extract containing 40 rat units per ml. Each ml. is equivalent to 2 mg. of 17-hydroxy-11-dehydrocorticosterone (Compound E) or 4 mg. of corticosterone.

† Generously supplied by the Upjohn Company, Kalamazoo, Michigan; The Abbott Laboratories, Chicago; Roche-Organon, Inc., Nutley, N. J.; Eli Lilly Company, Indianapolis, Ind.

TABLE 1

COMPARISON OF IONIC LEVELS IN PLASMA AND ERYTHROCYTES AND OF HEMATOCRIT AND PARASITEMIA VALUES IN GROUPS OF UNTREATED ANIMALS

GROUPS	PLASMA			ERYTHROCYTE			PLASMA Na + K	CELL Na + K	PLASMA Na/K	CELL Na/K	HEMATOCRIT	PARASITEMIA
	Na	K	Cl	Na	K	Cl						
I, n = 13												
Range	103–163	3.0–4.9	101–114	13.1–26.5	95–120	55–90	108–168	113–136	21.5–49.3	.111–.248	37.1–49.7	
Average	149	3.8	109	17.6	111	67	150	129	39.4	.160	41.8	
S. D.	5.2	1.8	3.9	3.7	7.4	8.8	15.2	5.9	7.1	.13	3.2	
S. E.	4.96	0.57	1.18	1.10	2.21	2.77	4.80	1.76	2.24	.039	0.92	
II, n = 14												
Range	137–174	3.3–5.5	96–114	14.6–27.1	99–120	51–91	142–182	126–140	29.1–51.2	.130–.263	23.0–38.0	+–17.6
Average	156	4.2	106	20.1	112	62	160	132	38.6	.181	32.5	3.8
S. D.	9.6	2.2	4.3	3.3	6.0	11.4	11.0	4.2	6.6	.044	4.2	4.6
S. E.	2.68	0.61	1.38	1.00	1.90	4.04	3.05	1.34	1.84	.015	1.18	1.38
III, n = 10												
Range	139–161	3.6–6.2	95–112	24.3–46.1	87–117	51–80	144–165	112–141	24.4–40.3	.208–.532	11.0–43.0	10.8–32.0
Average	150	4.7	103	30.7	104	69	155	131	33.2	.298	26.6	19.6
S. D.	7.9	0.70	4.6	7.1	10.2	9.4	8.0	8.5	4.8	0.10	8.5	6.4
S. E.	2.63	0.23	1.87	2.68	3.85	3.85	2.65	3.2	1.61	0.04	2.83	2.14
IV, n = 6												
Range	122–160	4.3–11.0	96–110	25.7–79.6	44–87		145–169	105–153	12.2–34.9	.298–3.13	6.9–26.8	45–123
Average	147	6.1	104	58.3	63		158	121	28.5	1.29	14.7	76
S. D.	4.6	2.5	1.8	11.2	24.3		5.4	17.3	8.5	1.0	6.9	25.4
S. E.	2.05	1.26	1.05	5.59	12.17		2.79	8.67	4.25	0.50	3.07	11.34

Group I, normal untreated animals
Group II, untreated animals, average of 124 hours after inoculation (range 92–140 hours)
Group III, untreated animals, average of 160 hours after inoculation (range 159–160 hours)
Group IV, untreated animals, average of 183 hours after inoculation (range 182–184 hours)
n indicates number of animals.

the malarious course was from 41.8 to 14.7 per cent while in the group treated with lipo-adrenal extract, the average reduction was somewhat more pronounced, *i.e.*, from 41.7 to 12.0 per cent (Tables 1–3 and Fig. 1). The average venous hematocrit reading of the untreated monkeys just before death was reduced 65 per cent from normal while that of the extract-treated group *at that time* had been reduced but 51 per cent. However, by the time the treated animals became moribund, the average hematocrit reduction was 71 per cent. Table 4 shows that both of these differences are statistically significant. Thus the hematocrit values of the treated animals are significantly higher than those of the untreated group at the time the animals in the latter group died. But they are also significantly lower than in the untreated group when the comparison is made at an equal

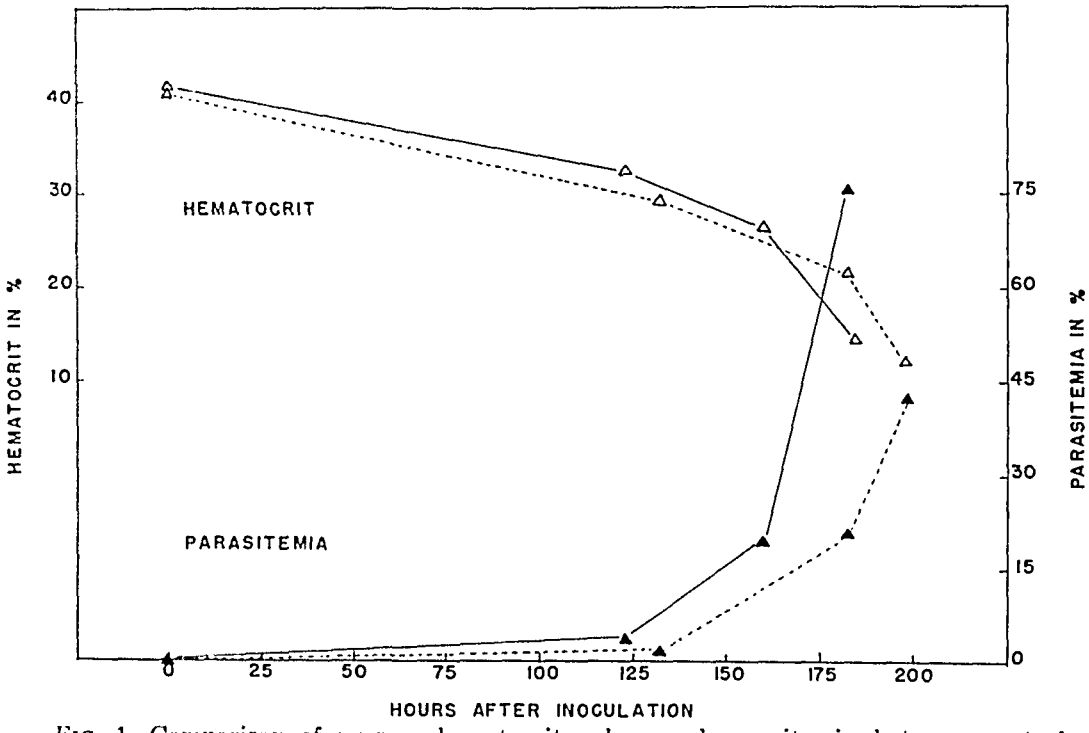


FIG. 1. Comparison of average hematocrit values and parasitemia between control animals (solid lines) and animals treated with lipo-adrenal extract (dotted lines).

number of hours before death (see Figure 1). Since the parasitemias of the untreated animals were consistently higher than those of the treated group, the greater final hematocrit reduction of the latter probably cannot be ascribed to a greater erythrocytic destruction. This postulate is further strengthened when plasma protein concentration changes of the two series are compared. The untreated group of animals showed a 0.6 Gm. per 100 ml. reduction in plasma protein (8 per cent decrease) over the malarious course while the extract-treated group had a 1.1 Gm. per 100 ml. reduction, amounting to a 15 per cent decrease from control values. Thus, it would appear that the hematocrit differences between the untreated and treated groups are due to greater water retention in the latter rather than differences in parasitic destruction of red cells.

TABLE 2

COMPARISON OF IONIC LEVELS IN PLASMA AND ERYTHROCYTES AND OF HEMATOCRIT AND PARASITEMIA VALUES IN GROUPS OF TREATED ANIMALS

GROUPS	PLASMA			ERYTHROCYTE			PLASMA Na + K	CELL Na + K	PLASMA Na/K	CELL Na/K	HEMATOCRIT	PARASITEMIA
	Na	K	Cl	Na	K	Cl						
A, n = 8												
Range	146–161	2.9–4.5	95–113	12.6–20.0	96–120	58–72	149–165	115–135	33.3–51.0	.113–.199	38.3–45.5	
Average	150	3.8	107	17.1	109	65	154	126	40.4	.159	41.7	
S. D.	2.4	1.7	5.7	2.1	7.3	5.2	4.7	5.2	5.6	0.22	2.5	
S. E.	0.92	0.66	2.14	0.79	2.74	1.95	1.76	1.95	2.10	0.09	0.96	
B, n = 8												
Range	137–161	3.1–4.6	101–112	16.5–29.1	86–114	52–67	140–165	111–133	33.4–44.8	.149–.292	23.2–33.8	+–8.4
Average	145	3.7	109	21.0	106	60	149	127	39.7	.202	29.2	2.0
S. D.	4.7	0.46	3.7	4.0	8.0	5.2	7.2	6.2	4.0	.046	3.0	2.5
S. E.	1.79	0.17	1.51	1.51	3.03	2.61	2.74	2.32	1.52	.017	1.14	0.93
C, n = 7												
Range	139–160	3.5–6.4	97–104	19.6–60.0	59–107		139–164	105–128	21.7–44.7	.183–1.017	11.1–36.1	7.5–60.6
Average	148	4.3	100	34.2	87		153	121	35.5	.440	23.1	21.3
S. D.	8.5	0.93	3.0	12.7	11.6		8.7	7.3	6.9	0.83	4.5	16.9
S. E.	3.46	0.39	2.12	5.02	4.71		3.54	2.97	2.83	0.34	1.84	6.89
D, n = 11												
Range	140–161	3.4–9.7	94–105	23.0–62.6	47–103	54–67	143–165	91–129	15.1–42.8	.247–1.124	4.9–20.0	6.1–89.0
Average	146	5.6	102	40.2	73	59	152	113	29.9	.603	12.0	42.6
S. D.	5.3	2.1	3.2	13.1	15.8	6.2	6.0	10.4	9.6	0.29	4.1	30.1
S. E.	1.67	0.71	1.14	4.62	5.59	4.35	1.97	3.69	3.21	0.10	1.30	9.52

Group A, normal animals, subsequently treated
Group B, treated animals, average of 126 hours after inoculation (range 92–142 hours)
Group C, treated animals, average of 182 hours after inoculation (range 168–192 hours)
Group D, treated animals, average of 199 hours after inoculation (range 197–200 hours)
n indicates number of animals.

Parasitemia. Other than differences in time of survival, perhaps the most striking clinical dissimilarity developed in these groups of animals is in the parasitemias (Fig. 1 and Tables 1, 2 and 4). It was found that at the time of death of the untreated animals, the latter had, on the average, a parasitemia of 75.5 per cent, while those receiving lipo-adrenal extract showed but a 19 per cent parasitemia. Indeed, even at the time the extract-treated animals succumbed, they had on the average a parasitemia of but 38.5 per cent, or approximately one-half the peak parasitemia of the control group.

Plasma ions. The reductions in plasma Na and Cl concentrations and the rise in plasma K illustrated in Figure 2 and Tables 1 and 2 are qualitatively similar to those previously reported.[13, 17] The greatest percentage alteration occurred

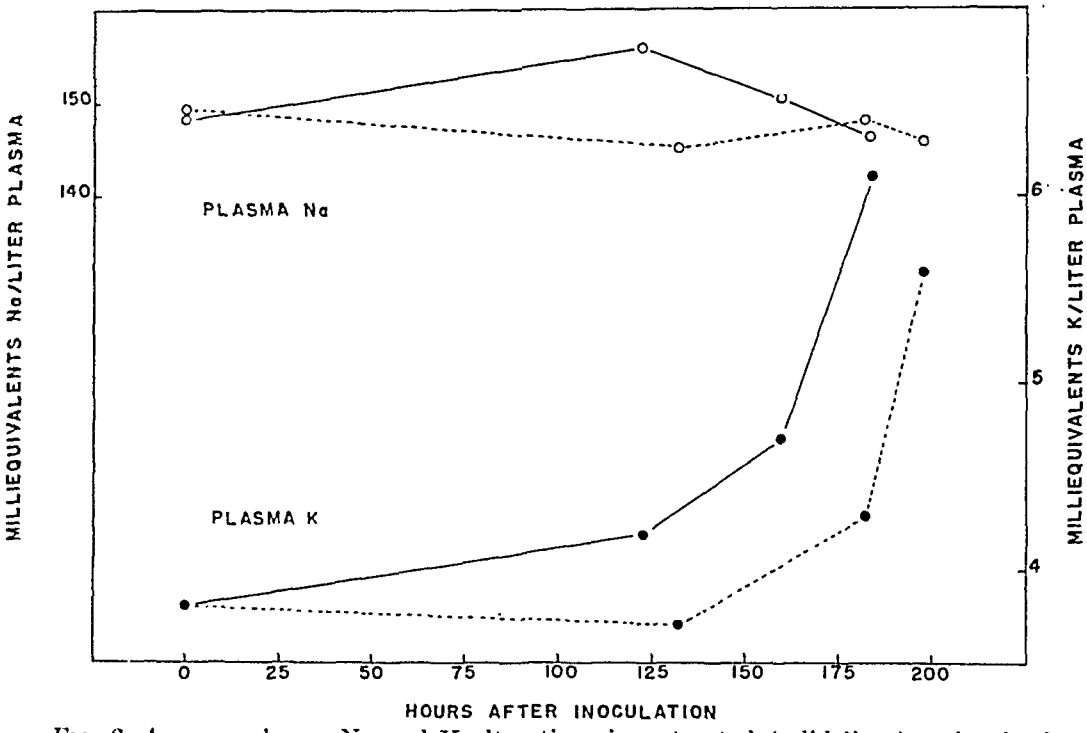


FIG. 2. Average plasma Na and K alterations in untreated (solid lines) and animals treated with lipo-adrenal extract (dotted lines).

in the plasma K concentration. At the time the untreated animals died, their plasma K averaged 6.1 mEq./L. (a 61 per cent increase over control values) while measurements made on the treated group at that time revealed a plasma K level of but 4.5 mEq./L. (an 18 per cent increase over controls). Even at the time of their death, the plasma K concentration of the extract-treated animals had risen to but 5.6 mEq./L. which constitutes a 47 per cent increase over normal values. As might be expected, therefore, differences likewise appear in the sodium-potassium ratio illustrated in Figure 4.

Erythrocyte ions. As previously reported,[13] striking alterations in cellular ionic anatomy occur during the course of *P. knowlesi* infections in monkeys. While the dosage of adrenal extract selected did not alter the direction of ion movements

across the membrane of the unparasitized erythrocyte, quantitative differences did appear in such shifts (Fig. 3). Thus at the time of death, the untreated controls showed an average cell Na level of 58 mEq./L. which constitutes a 229 per cent increase over normal values. At this time, the extract-treated groups had an average cell Na of 34 mEq./L. (a 99 per cent increase from normal) and even at the time of death had a cell Na concentration of but 40 mEq./L. Thus it would seem that lipo-adrenal extract treatment reduced the tendency for Na to enter cells and for K to be removed from cells.

Reference to Figure 4 will illustrate one further point regarding cellular ionic differences between the two groups of animals. It can be seen that while the Na/K ratio within erythrocytes is not different in the two series early in the course of the disease, that the untreated group shows a marked terminal rise in this ratio to a value exceeding unity. While such a pronounced rise in Na/K

TABLE 3

STATISTICAL EVALUATION OF CHANGES IN IONIC CONCENTRATIONS AND HEMATOCRIT VALUES FROM CONTROL LEVELS WITHIN UNTREATED AND TREATED GROUPS (t VALUES)

BETWEEN GROUPS*	PLASMA			CELL			PLASMA Na + K	CELL Na + K	PLASMA Na/K	CELL Na/K	HEMATOCRIT
	Na	K	Cl	Na	K	Cl					
I and II	1.3	.48	1.7	1.7	.24	1.0	1.7	1.4	.28	.51	6.2[1]
I and III	.30	1.5	2.8[1]	4.5[1]	1.7	.40	.86	.66	2.2	2.4	5.1[1]
I and IV	.35	1.7	3.0[1]	7.2[1]	3.9[1]	—	1.4	.81	2.3[2]	2.3[1]	8.4[1]
A and B	2.4[2]	.14	.76	2.3[2]	.73	1.7	1.5	.30	.27	.50	8.4[1]
A and C	.50	.65	2.3[2]	3.4[1]	4.0[1]	—	.30	1.3	1.4	.80	8.9[1]
A and D	2.1[2]	1.9	2.1	4.9[1]	5.7[1]	1.3	.75	3.0[1]	2.7[2]	3.4[1]	18.3[1]

* See footnote Tables 1 and 2

[1] Significant at the 1 per cent level or less

[2] Significant at the 1 to 5 per cent level

values was not found in all animals, at least one, terminally untreated, had three times as much Na as K in the erythrocytes (Na/K ratio, 3:1). On the other hand, the lipo-adrenal extract treated group is characterized by a terminal average value for the Na/K ratio of but 0.6. Even in this group, however, two animals showed "mild" reversals of Na/K ratios, *i.e.*, values of 1.0 and 1.1, respectively.

These data are reported to illustrate the severity of alteration in the cellular distribution of these ions. That these phenomena are not limited to the erythrocytic membrane is borne out by preliminary observations of ionic concentrations in tissues. Large increments in the amount of Na in liver have been previously observed in this laboratory in animals dying of untreated *P. knowlesi* infections. Concomitant loss of K from tissues has also been seen. Indeed, in animals developing overwhelming parasitemia, actual reversal of the Na/K ratio in liver has been found, although such severe changes have not been observed in all animals coming to autopsy.

As a result of these observations, therefore, tissue ionic studies have been made in a few pairs of animals, one being an untreated control and the other an identically infected animal receiving daily doses of lipo-adrenal extract. In such a pair, muscle analysis revealed the following: tissue water, 78 per cent in both animals; tissue Na, an increase of 18 mM/1000 Gm. of wet tissue in untreated over the treated animal; tissue K, a decrease of 29 mM/1000 Gm. wet tissue in untreated over the extract-treated animal. In brain and liver tissue similar differences were found even though the plasma K of the untreated animal was higher and the plasma Na lower than that of the extract-treated monkey. Thus it would appear that permeability alterations observed in erythrocytes are, in this disease, a reasonable qualitative reflection of alterations in the permeability of tissue cells.

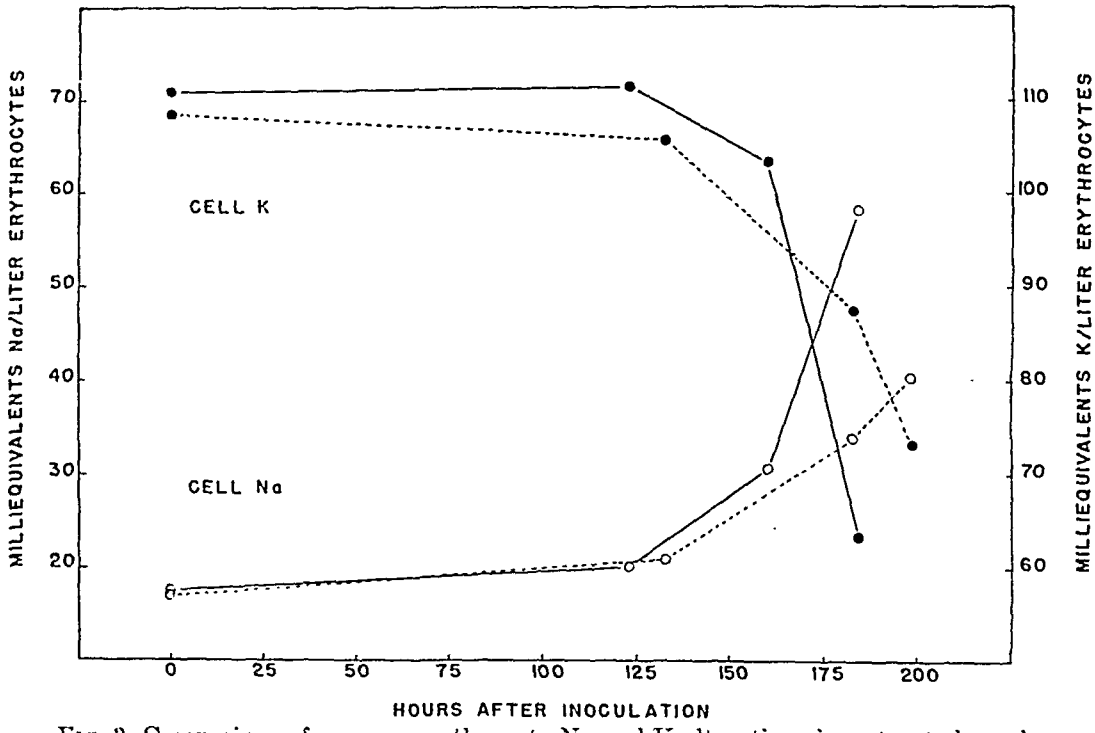


FIG. 3. Comparison of average erythrocyte Na and K alterations in untreated monkeys (solid lines) and in animals treated with lipo-adrenal extract (dotted lines).

DISCUSSION

The strong inferential evidence of general alteration in cellular permeability to ions in malaria from the studies of sulfocyanide (SCN) distribution in both infected monkeys and in man and the more direct and specific evidence of this phenomenon relating to Na and K distribution in the uninvaded erythrocyte and in tissue cells, requires the establishment of at least an hypothesis of explanation.

The reported directional changes in ion movement in malaria, *i.e.*, Na entrance into cells and K loss from cells, is qualitatively identical to ionic alterations which can be produced in normal animals by prolonged treatment with desoxycorticosterone acetate (DCA, the so-called "salt-and-water-balance" hormone

of the adrenal cortex). Similarly the renal conservation of Na and Cl and the renal loss of K in malaria is very nearly the same as that which can be produced by DCA treatment. It did not seem rational, therefore, to administer this particular adrenal cortical steroid in any attempt at amelioration of the observed changes.

On the other hand, the morphologic evidence of a severe adrenal cortical lesion in fulminating malaria (both simian and human) is overwhelming. It occurred to us, therefore, that these permeability alterations might be caused (at least in part) by a functional imbalance between the amount of circulating desoxycorticosterone and the so-called "carbohydrate-active steroids" of the adrenal cortex (the latter being represented in our investigations by lipo-adrenal extract). The experiments reported here are to be considered a preliminary test of this general hypothesis and the results are encouraging, if inconclusive. It is possible that the employment of a different dosage would provide even more

TABLE 4

STATISTICAL COMPARISON (t VALUES) OF IONIC CONCENTRATION AND HEMATOCRIT VALUES OF CORRESPONDING GROUPS OF TREATED AND UNTREATED ANIMALS

BETWEEN GROUPS*	PLASMA			CELL			PLASMA Na + K	CELL Na + K	PLASMA Na/K	CELL Na/K	HEMATOCRIT	PARASITEMIA
	Na	K	Cl	Na	K	Cl						
I and A	.24	0	.90	.37	.65	.65	.67	1.0	.33	.01	.75	—
II and B	3.4[1]	.79	1.6	.49	1.7	.54	2.8[1]	1.9	.46	.09	2.0	1.1
III and C	.53	.89	1.0	.62	2.7[2]	—	.57	2.2[2]	.71	.42	1.0	.24
IV and C	.32	1.36	1.86	3.21[1]	1.85	—	1.20	0	1.40	1.40	2.35[2]	4.09[1]
IV and D	.34	.34	1.6	2.5[2]	.75	—	1.8	.87	.26	1.3	.81	2.2[2]

* See footnote, Tables 1 and 2

[1] Significant at the 1 per cent level or less

[2] Significant at the 1 to 5 per cent level

definite amelioration of the biochemical and clinical manifestations of simian malaria.

The facts that each extract-treated animal outlived his controls and extract treatment uniformly resulted in significantly lower parasitemias seem to indicate that true clinical as well as chemical amelioration can be expected from this regimen.

It has been reported by Harrop and Benedict,[7] and confirmed in this laboratory, that rather pronounced changes in ionic balance and renal ionic excretion can be induced in animals and in man by administration of insulin. During the hypoglycemic phase following insulin we have found large increments in erythrocyte Na, for instance, and the pattern of urinary excretion of ions in insulin hypoglycemia is quite similar to that previously reported in simian malaria,[10] *i.e.*, a reduction of Na and Cl in urine and a concomitant increase in K excretion.

These facts, together with numerous observations of ionic shifts in patients in diabetic coma, argue that serious derangements in carbohydrate metabolism

are associated with mineral imbalances which are, in general, opposite in direction to those seen in total adrenal insufficiency or in DCA insufficiency. It is, therefore, our belief that the normal pattern of ionic distribution depends upon a balance between the two major functional groups of steroids of the adrenal cortex. It is postulated that when a hormonal imbalance occurs which favors DCA ascendency, one type of ionic pattern appears (renal retention and cellular entrance of Na and Cl and cellular and renal loss of K) and that when the hormonal imbalance is characterized by a *relative* reduction in DCA that the opposite ionic pattern (cellular and renal loss of Na and Cl and K retention) can be expected. The implications of this hypothesis in the treatment of those clinical entities characterized by "hypoadrenalism" are obvious.

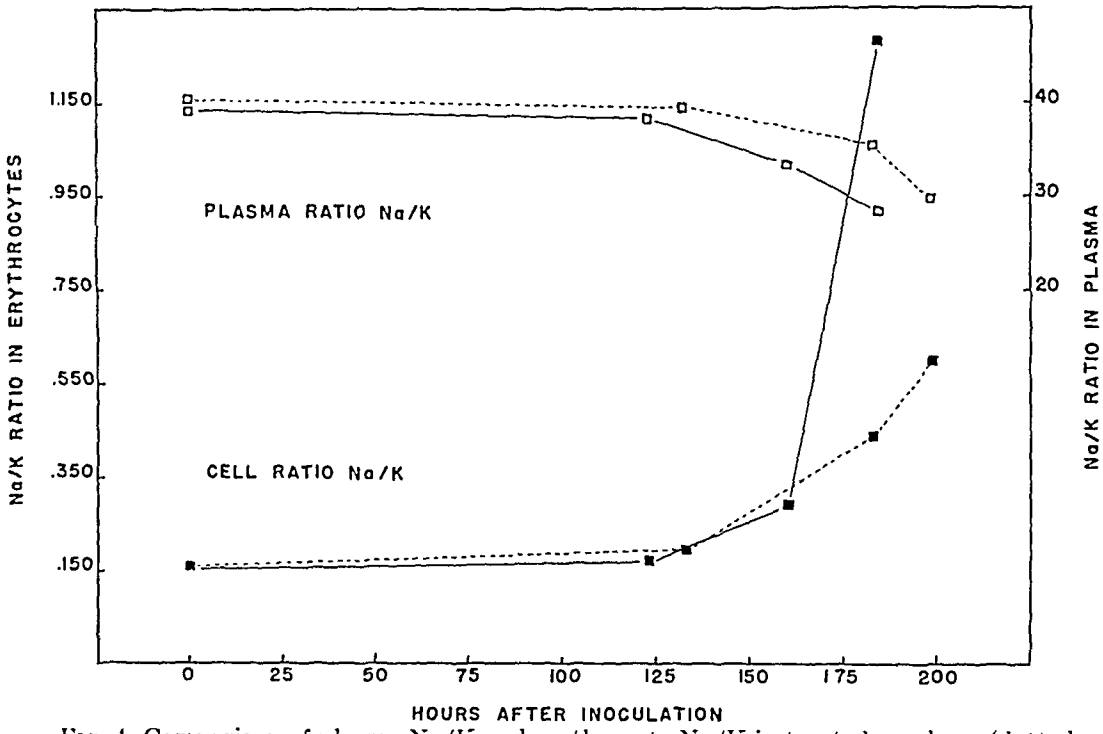


FIG. 4. Comparison of plasma Na/K and erythrocyte Na/K in treated monkeys (dotted lines) and in untreated monkeys (solid lines).

SUMMARY

The following differences were observed between two groups of monkeys identically infected with *Plasmodium knowlesi*, one of which received daily injections of 5 ml. of lipo-adrenal extract:

1. The extract-treated animals uniformly outlived their controls.

2. The control animals showed earlier development of high parasitemias which attained average values 100 per cent greater than the extract-treated animals.

3. Sodium entrance into erythrocytes and tissue cells and loss of potassium from cells was significantly reduced in the treated group.

4. Our results indicate that ionic distribution in the normal subject depends

upon a functional balance between the so-called "salt-and-water-balance" type and the "carbohydrate-active" type of steroids of the adrenal cortex.

REFERENCES

1. Davis, A. K., and Overman, R. R.: Internal standard flame photometric analysis of biological materials. To be published.
2. Dudgeon, L. S., and Clarke, C.: A contribution to the microscopical histology of malaria, as occurring in the Salonika Force in 1916, and a comparison of these findings with certain clinical phenomena. Lancet, **2:** 153–156, 1917.
3. Fraga, A.: Insuficiencia pararenal no impaludismo; contribuicao ao seu estudo anatomo-clinico. Dissertation. (Quoted by Flosi, 1944.)
4. Golden, A.: Adrenal gland lesions in experimental simian malaria and similar human lesions in varied diseases. Federation Proc., **7:** 271, 1948.
5. Golden, A., and Overman, R. R.: Sodium-potassium levels and adrenal gland necrosis in experimental simian malaria. Federation Proc., **7:** 270–271, 1948.
6. Golden, A., and Overman, R. R.: Experimental cortical necrosis of the adrenal gland in the monkey in diphtheria and in malaria. Am. J. Clin. Path., **19:**899–906, 1949.
7. Harrop, G. A., and Benedict, E. M.: The participation of inorganic substances in carbohydrate metabolism. J. Biol. Chem., **59:** 683–697, 1924.
8. Maegraith, B.: Pathological Processes in Malaria and Blackwater Fever. Oxford: Blackwell Scientific Publication, pp. 321–335, 1948.
9. Natali, C.: Ricerche istologiche sulle alterazioni delle surrenali in um caso di malaria perniciosa e ricerche comparative con quelle delle surrenali di scimme sperimentalmente infettate (Plasmodium knowlesi). Riv. di malariol., **13:** 563–584, 1934.
10. Overman, R. R.: Permeability alterations in disease. J. Lab. and Clin. Med., **31:** 1170–1173, 1946.
11. Overman, R. R.: Reversible permeability alterations in febrile disease. Communications, 17th Int. Physiol. Cong., Oxford, XVII: 93–94, 1947.
12. Overman, R. R.: Reversible permeability alterations in the erythrocytes of the malarious monkey. Federation Proc., **6:** 174–175, 1947.
13. Overman, R. R.: Reversible cellular permeability alterations in disease: *in vivo* studies on sodium, potassium, and chloride concentrations in erythrocytes of the malarious monkey. Am J. Physiol., **152:** 113–121, 1948.
14. Overman, R. R., and Davis, A. K.: The application of flame photometry to sodium and potassium determinations in biological fluids. J. Biol. Chem., **168:** 641–649, 1947.
15. Overman, R. R., Davis, A. K., and Tharp, E.: Blood and extracellular fluid volumes and ionic balance in human therapeutic malaria. Federation Proc., **6:** 174, 1947.
16. Overman, R. R., and Feldman, H. A.: Effect of fatal *P. knowlesi* on simian circulatory and body fluid compartment physiology. J. Clin. Investigation, **26:** 1049–1054, 1947.
17. Overman, R. R., Hill, T. S., and Wong, Y. T.: Physiological studies in the human malarial host. I. Blood, plasma, "extracellular" fluid volumes and ionic balance in therapeutic *P. vivax* and *P. falciparum* infections. J. Nat. Malaria Soc., **8:** 14–31, 1949.
18. Paisseau, G., and Lemaire, H.: De l'insufficance surrenale dans le paludisme. Pr. med., **24:** 547, 1916.
19. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry. Baltimore: Williams and Wilkins, pp. 829–847, 1932.
20. Phillips, R. A., Van Slyke, D. D., Emerson, K., Jr., Hamilton, P. B., and Archibald, R. M.: Copper sulfate method for measuring specific gravities of whole blood and plasma. Bull. U. S. Army M. Dept., **71:** 66–83, 1943.

III. CORTICAL NECROSIS OF THE ADRENAL GLAND IN MAN

Evolution and Varieties of Acute Necrosis and Its Repair*

ALFRED GOLDEN, M.D.†

From the Pathological Institute of the University of Tennessee College of Medicine, Memphis, Tennessee

The acute stage of necrosis of the adrenal cortex was described by Golden and Overman[3] in experimental monkey malaria. It was also shown by Overman, Bass, Davis, and Golden[5] that certain biochemical alterations were associated with this lesion. A similar lesion in human disease was demonstrated by Rich,[6] Kinsman, D'Alonzo and Russi,[4] and Golden.[1] It is the purpose of this report to survey the varying degrees and types of adrenal cortical injury as seen in human subjects postmortem.

PROCEDURE

Inasmuch as this form of adrenal cortical necrosis may be obscured by postmortem autolytic changes, the cases to be reported are limited to those in which the glands were removed and placed in fixative solutions (usually 10 per cent formaldehyde) within one or two hours after death. Fifteen such cases were selected to illustrate the varying degrees and forms of this lesion. It will be noted that a wide variety of diseases is represented in which this lesion appears. It is not the purpose of this paper to discover a basic morbid process or abnormality, biochemical or otherwise, common to these diseases. The cases were selected in order to trace the evolution of the lesion from the acute stage, including those accompanied by acute and chronic inflammation.

REPORTS OF CASES

I. *Cases with Variable Degrees of Necrosis of Adrenal Cortex*

Case 1

A 46-66. A 26 year old white man entered the hospital four hours after sustaining multiple gunshot wounds of the abdomen. He was operated upon immediately, several portions of the bowel were resected, and numerous perforations were repaired by purse-string sutures. It was necessary to re-operate three to four days later because of extravasation of urine, at which time a catheter was introduced suprapubically as well as through the urethra. The patient expired approximately one week after the second operation. The chief findings at autopsy were multiple surgically-repaired gunshot wounds of the gastrointestinal tract, bilateral early bronchopneumonia, slight central necrosis of the liver and acute tubular necrosis with pigmented casts in the kidneys (hemoglobinuric nephrosis; lower nephron syndrome). Microscopically, the cortex of the adrenal glands showed numerous pale foci of necrosis (Fig. 1). The lesion was in all respects similar to that described in experimental monkey malaria.[3] It consisted substantially of a disruption of the continuity of the adrenal

* Presented, in part, before The Society for Experimental Pathology, 1947.[2]
Received for publication, June 6, 1949.

† Present address: Veterans Administration Hospital, Batavia, New York.

cords by simple cellular solution and the formation of lacunae or pseudotubules. In none of the many foci of necrosis was there any hemorrhage or leukocytic infiltration. The lesions were limited to the zona fasciculata.

Case 2

A 47-75. A 19 year old woman was admitted to the hospital in coma, with fever, pounding pulse, heavy respiration, rigid neck, fixed pupils, flat optic discs, and paralysis of the lower extremities. She died within 24 hours. At autopsy the following changes characteristic of acute *Plasmodium falciparum* malaria were found: splenomegaly (410 Gm.), the spleen having a thin capsule and dark, almost black pulp; an enlarged liver with a grayish cast; bilateral bronchopneumonia; and numerous congested and hemorrhagic foci in the brain. Microscopically, the lesions were those of acute, overwhelming, malarial infection with malarial pigmentation in the spleen, liver and lymph nodes, and plugging of capillaries of the brain with conglutinated, parasitized erythrocytes. Special stains demonstrated the characteristic forms of *P. falciparum*. On microscopic examination of the adrenal glands, numerous foci of bland necrosis were observed in the zona fasciculata resulting in the lacuna formation described above (Fig. 2). This case was referred to in a previous publication.[3] Further sections demonstrated areas of frank, polymorphonuclear leukocytic infiltration in association with the areas of necrosis. Slight interstitial leukocytic infiltration is demonstrable in Figure 2.

Case 3

A-7669. A 57 year old white man was admitted to the hospital with a clinical diagnosis of bilateral lobar pneumonia which was confirmed at autopsy. Bacteriologic studies were not completed. Microscopically, the adrenal cortex showed numerous foci of a *coagulative* type of necrosis in which the contour of the cells was maintained but nuclear staining and differentiation were lost (Fig. 3). It will be noted that the lesion was accompanied by mild interstitial mononuclear infiltration. Lacuna formation among cells with pyknotic nuclei alternated with broad patches of the coagulative type of necrosis. It was estimated by "step sections" that about one third of the cortical volume was involved in this combination of lesions. There was no evidence of hemorrhage grossly or microscopically.

The changes described in the above 3 cases were not accompanied by visible gross changes.

II. *Cases with Combination of Necrosis and Leukocytic Reaction in Adrenal Cortex*

Case 4

A 46-69. A 72 year old white man was hospitalized for prostatic resection because of glandular hyperplasia of the prostate, following which he developed acute gangrenous cystitis and bilateral gangrenous pyelonephritis. He died 12 days after the operation and the clinical findings were confirmed at autopsy. The hemorrhagic necrotic exudate extended from the capsule of the kidney to the perirenal and periadrenal fat on each side. Microscopically, the cortex of the adrenal glands showed many areas of well delineated anemic necrosis bordered by thin but definite zones of polymorphonuclear leukocytic reaction (Fig. 4). No direct continuity between these zones of cortical necrosis and of surrounding hemorrhage and necrosis in periadrenal adipose tissue could be demonstrated in numerous sections.

In Case 5 essentially similar lesions of the adrenal cortex were seen without involvement of the periadrenal adipose tissue or of any structures adjacent to the adrenal gland.

Case 5

A 48–35. A 72 year old white man was admitted to the hospital with a diagnosis of chronic cholecystitis for which cholecystostomy was performed. He died shortly after the operation and at autopsy he was also found to have moderately extensive and active apical pulmonary tuberculosis. On microscopic examination, the cortex of both adrenals revealed several discrete patches of anemic necrosis in the zona fasciculata, bordered by a thin but definite line of leukocytic reaction (Fig. 5), changes essentially similar to those in the preceding case.

A more intense leukocytic reaction in association with this lesion is illustrated in Case 6.

Case 6

A 48–828. A 35 year old Negro man injured his right hand three weeks before being admitted to the hospital. The injury required amputation of the fourth and fifth fingers, at which time tetanus antitoxin (1500 units) was administered. This did not prevent the development of severe clinical tetanus three days prior to hospitalization and about 18 days after injury. He died on the fourth hospital day. Therapy consisted of tetanus antitoxin, sedatives, and penicillin. At autopsy bilateral aspiration bronchopneumonia was found. Microscopic examination of the adrenal glands revealed bilateral extensive, patchy cortical necrosis bordered by relatively intense leukocytic reaction (Fig. 6).

The anemic type of necrosis in these cases (Figs. 4–6) was bordered by polymorphonuclear leukocytes and monocytes, and relatively few lymphocytes. This type of necrosis did not exist independently since other portions of the cortex of the adrenal glands showed extensive necrosis resulting in pseudotubule formation identical to that of the first three cases.

A slightly different form of acute necrosis with inflammation of the adrenal cortex is demonstrated in Case 7.

Case 7

A 48–532. A 42 year old Negro woman was admitted from the outpatient service with a history of fulminant hypertension. She died five days later in uremia. At autopsy there was severe arteriosclerosis of the intrinsic renal circulation along with scattered foci of acute necrotizing arteriolitis chiefly in the kidneys, heart, pancreas and spleen. The heart weighed 440 Gm. and was consistent with hypertensive hypertrophy. No infectious process was discovered. Microscopically in the adrenal cortex, there were numerous areas of necrosis and dense infiltration with, rather than a border of, polymorphonuclear leukocytes, small

NOTE: All the photomicrographs are of the adrenal cortex and all sections are stained by hematoxylin and eosin.

FIG. 1. From a patient who died after multiple gunshot wounds of the abdomen. The lesion seen at A was one of a number of foci limited to the zona fasciculata and characterized by disruption of the continuity of the cortical cords. Defects or lacunae were surrounded by intact cells. No leukocytic reaction was present. × 110.

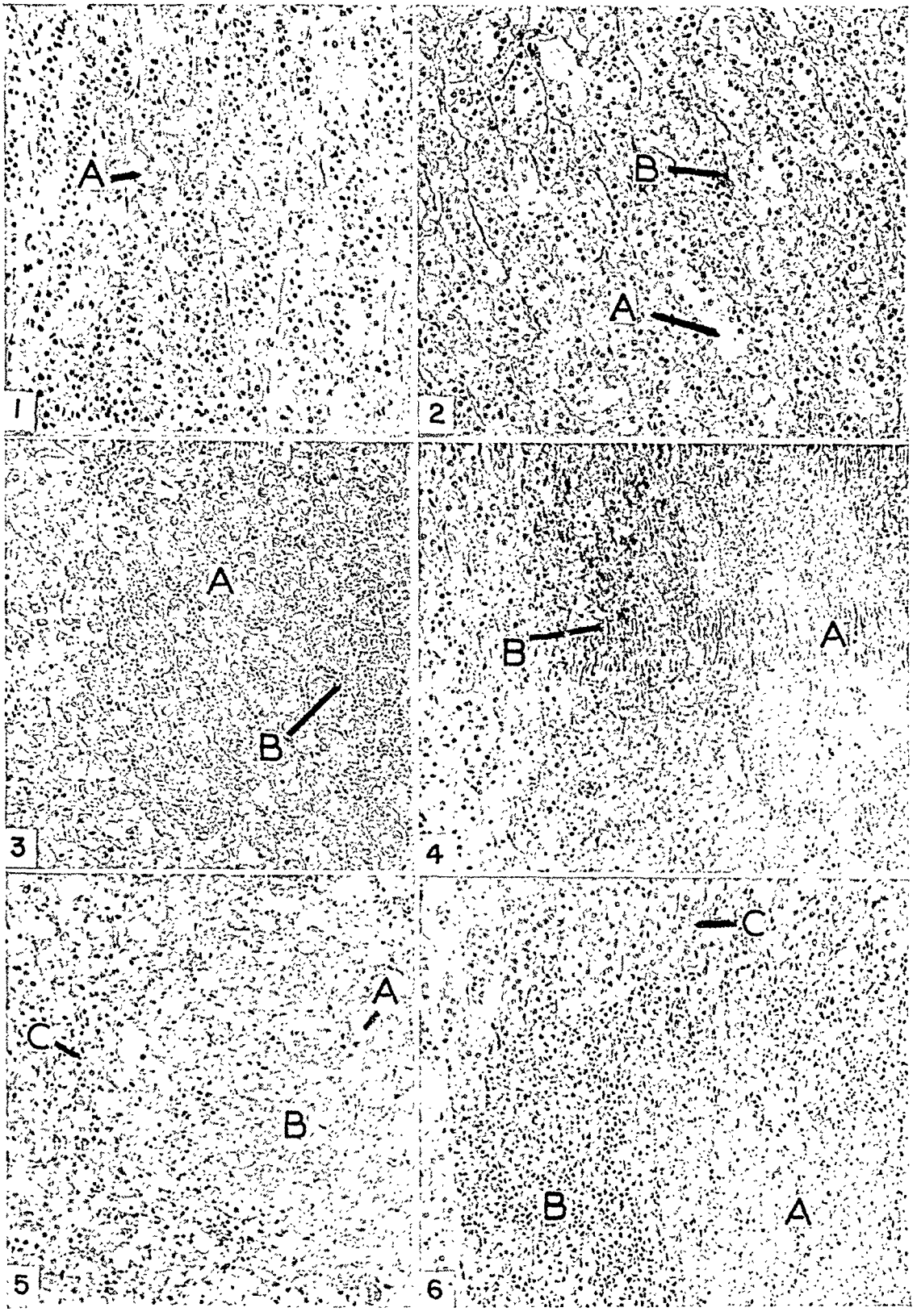
FIG. 2. From a patient with acute overwhelming *P. faliciparum* infection. The lacuna or pseudotubule formation (A) is more advanced than in the preceding case. Early polymorphonuclear leukocytic infiltration is seen at B. × 110.

FIG. 3. Coagulative type of necrosis. Case of lobar pneumonia. The cellular contours are maintained but cytologic detail is obscure (A). Early leukocytic reaction is seen interstitially (B). Adjacent areas showed lesions identical to those of Figures 1 and 2. × 110.

FIG. 4. From a patient with acute gangrenous cystitis and pyelonephritis. Large patches of anemic necrosis at A are bordered by thin zones of leukocytic reaction at B. × 110.

FIG. 5. The shadowy outlines of preexisting lacuna formation of the zona fasciculata are seen at A. The surrounding cells are completely necrotic (B). There is no hemorrhage. The periphery of the necrotic patch is occupied by an irregular line of leukocytes (C). × 110.

FIG. 6. The large patch of anemic necrosis (A) is bordered by a thick layer of polymorphonuclear and mononuclear phagocytes (B). Lymphocytes are present in small numbers. Plasma cells are seen only occasionally. Pseudotuble formation is seen at C. × 110.



FIGS. 1-6

numbers of monocytes and only occasional lymphocytes and eosinophils (Fig. 7). Adjacent to these areas of mixed necrosis and leukocytic infiltration in the zona fasciculata there were also small zones of nonpurulent necrosis resulting in lacuna-formation. Necrotizing arteriolitis, which was present in sections of the kidneys, was not demonstrable in any of the large number of "step sections" of the adrenal glands. There was no evidence that these adrenal lesions were secondary to local vascular disturbances. They resembled in every respect those described in the above 2 cases. Figure 8 represents a different field under higher magnification and shows the type of necrotic lesion of the preceding 3 cases.

III. *Cases with Hemorrhagic Necrosis of Adrenal Cortex*

Case 8

A 46-60. A 24 year old white woman was admitted to the hospital with acute meningococcic meningitis. The diagnosis was confirmed at autopsy three days later. Grossly, the adrenal glands were hemorrhagic. Microscopically, they showed advanced hemorrhagic necrosis involving the major portion of the cortex bilaterally (Figs. 9 and 10). It will be noted that this type of adrenal gland necrosis resulted in the formation of wide lacunae which differed from those described above (*e.g.*, in Fig. 2) only in the extensive hemorrhagic infiltration into them. In this case the general contour of the adrenal cords was more or less retained and outlined by the sinusoidal meshwork. No zones of leukocytic reaction were found in this case in many "step sections". Extensive but patchy and occasionally minute foci of necrosis of the sinusoidal reticulum were demonstrable by special staining methods.

Case 9

A–6003. A 16 month old Negro male child died in the receiving ward of the hospital. He had a scaly rash on the face, trunk and arms, which was diagnosed as measles. Autopsy revealed acute interstitial pneumonitis compatible with that accompanying measles. (Blocks of skin prepared for microscopic examination were not suitable for diagnosis because of technical difficulties.) Grossly, the adrenal glands were engorged with blood; microscopically they showed severe hemorrhagic necrosis (Fig. 11).

These cases illustrate the relationship that exists between necrosis, the formation of lacunae, and hemorrhagic necrosis. In the second case this lesion had progressed a little, since leukocytic reaction was present.

A slightly different form of hemorrhagic necrosis of the adrenal cortex is demonstrated in Case 10.

Case 10

A–8095. A Negro child died with severe pyelonephritis shortly after admission to the hospital. At autopsy, grossly and microscopically, there was found widespread hemorrhagic necrosis of the adrenal cortices (Figs. 12 and 13). Congestion of the sinusoids was prominent.

FIG. 7. The lesion which was but one of a number seen in a patient with fulminant hypertension showed a zone of necrosis (A). Note that the area is heavily infiltrated by polymorphonuclear leukocytes (B). Intact cells are seen at C. × 110.

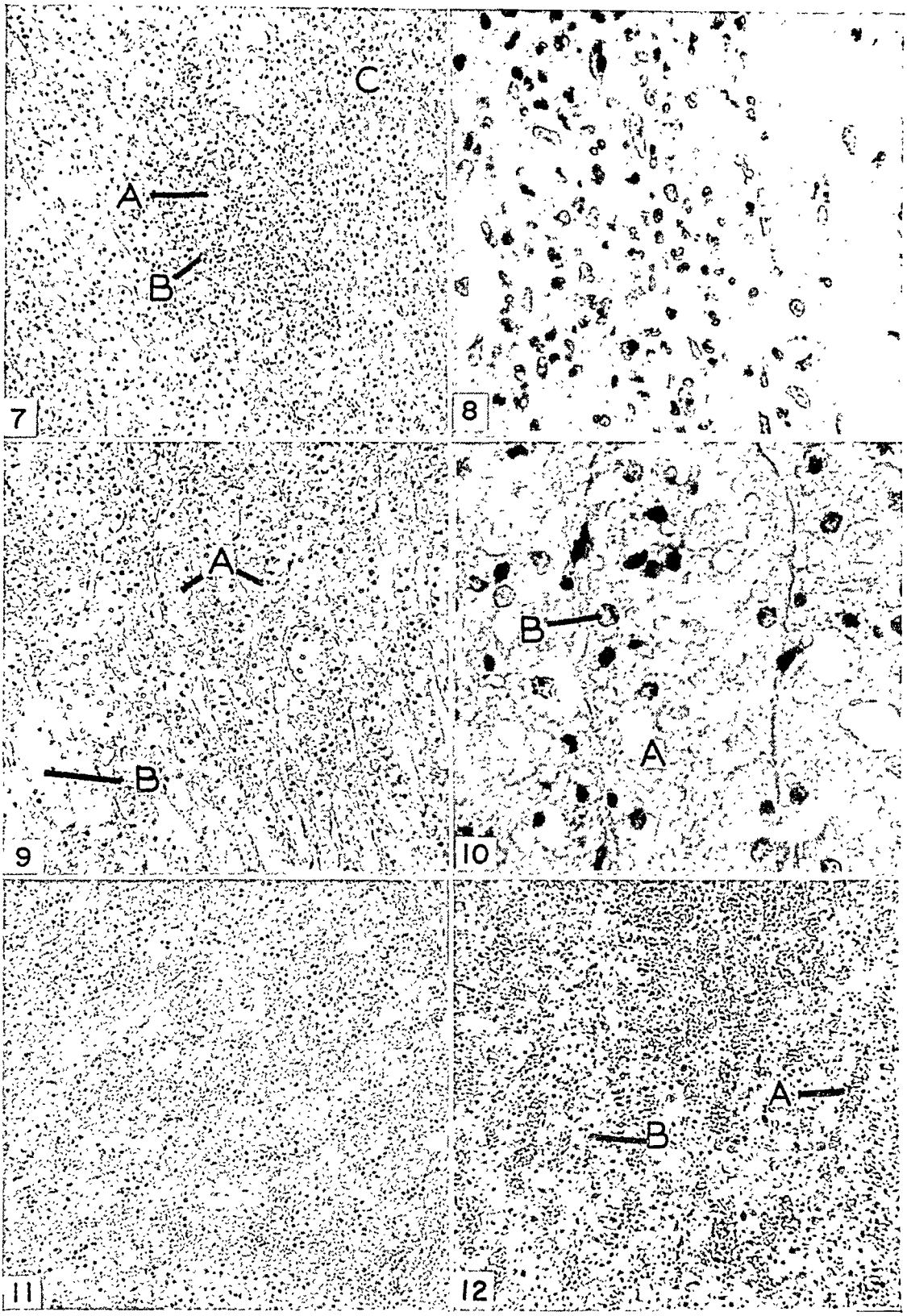
FIG. 8. This is a high power view of another portion of the zona fasciculata from the same case as Figure 7, showing an area of anemic necrosis bordered by leukocytes. × 440.

FIG. 9. From a patient with meningococcic meningitis. Note the great destruction of cortical cells (A) and the extensive hemorrhagic infiltration (B). × 110.

FIG. 10. High power view from same case as Figure 9, showing closely packed erythrocytes (A) in a preexisting zone of pseudotubule formation. Note that only few cortical cells (B) are identifiable, the remainder having undergone dissolution. × 440.

FIG. 11. Extensive hemorrhagic necrosis of the zona fasciculata in an infant that died of acute interstitial pneumonitis, probably following measles. × 110.

FIG. 12. From a child who died of severe pyelonephritis. Hemorrhagic cortical necrosis of adrenal glands was seen grossly. The hemorrhage is less marked than in the preceding case but the pooling of blood in the sinusoids (A) is profound. Note that the continuity of the adrenal gland cords (B) has been disrupted completely. × 110.



FIGS. 7-12

Apparently sufficient reticulum meshwork was preserved in this case to prevent the outpouring of all of the contained blood into the surrounding necrotic tissue. The great necrosis and separation of the cellular elements is well illustrated in Figure 13 along with early but definite interstitial leukocytic infiltration.

Case 11

A 48–423. For two weeks before hospital admission a 40 year old man took an undetermined amount of sulfadiazine by self-medication. There ensued weakness, dizziness, and painful masses in the submandibular area. The blood findings were those of agranulocytosis. He had a fever, elevated pulse rate, and an ulceration of the left tonsillar area covered with necrotic membrane. At autopsy the bone marrow showed myeloid hyperplasia with maturation arrest, consistent with agranulocytosis. Sulfadiazine crystals were demonstrable in frozen sections of the kidneys. Paraffin-imbedded sections of the kidney showed advanced bilateral acute tubular necrosis of the distal as well as the proximal portions of the nephrons (lower nephron syndrome; hemoglobinuric nephrosis), and acute patchy necrosis of the liver substance with hemorrhages into them. There were widespread patchy necrotic areas in the cortex of the adrenal glands characterized by wide lacunae heavily infiltrated with intact erythrocytes. The microscopic appearance of a typical zone is shown in Figure 14.

IV. *Case with Focal Hemosiderin Pigmentation and Associated Chronic Inflammation in Adrenal Cortex*

Case 12

A–8067. A 60 year old Negro man had the clinical and serologic findings of general paresis. At autopsy the microscopic findings were consistent with paresis. There was an active syphilitic aortitis, confluent bronchopneumonia of three lobes, a lung abscess, and a nonobstructing annular adenocarcinoma of the descending colon. These lesions were obviously of more than a few days' duration. A search of the record revealed no known previous illness. Decubital ulcers of the sacral regions and of the trochanteric areas were known to have been of about one week's duration. The patient revealed no evidence of acute infectious disease in the past year, but he was not under the care of a physician at any time during this period. One month before his final hospital admission he had acute urinary obstruction on the basis of prostatic hypertrophy and required catheterization.

The adrenal cortex microscopically showed patchy defects in the continuity of the cords, chiefly in the zona fasciculata, occasionally in the zona glomerulosa, and rarely in the zona reticularis. The spaces previously occupied by adrenal cells were filled with coagulated material, presumably protein, which appeared as tiny fluffy masses, occasionally in shreds; the spaces were liberally infiltrated with plasma cells and lesser numbers of mononuclear

FIG. 13. High power view of Figure 12, showing disruption of the cords of the zona fasciculata. Shadowy outlines of some cells can be seen at A. Masses of ragged cytoplasm containing pyknotic nuclei are seen at B. Hemorrhagic infiltration is seen at C. Individual cells, no longer in cord formation, are seen at D. Leukocytic infiltration (E) is sparse. × 440.

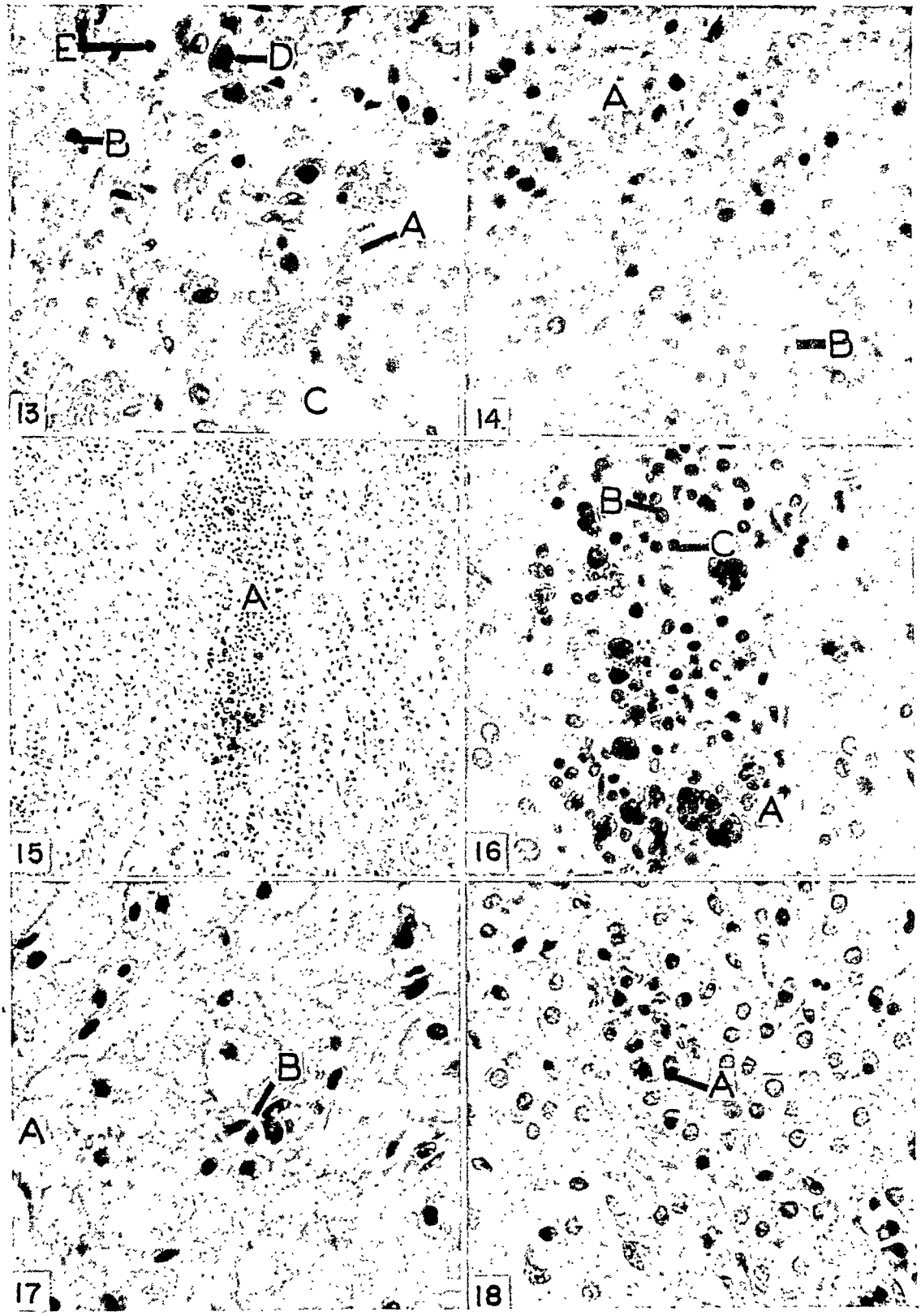
FIG. 14. From a patient with sulfadiazine poisoning. The gland cords are still fairly well outlined. Wide lacunae or pseudotubules are seen at A filled with erythrocytes (B). Many of the cells of the zona fasciculata have disappeared as is indicated by the sparseness of nuclei. × 440.

FIG. 15. From a patient with paresis. One of a number of defects in the adrenal cortex is seen at A. The photograph does not show the background of coagulated eosinophilic substance, presumably protein material. The nature of the infiltration is seen in detail in the next figure. × 110.

FIG. 16. High power of Figure 15. Hemosiderin-laden phagocytes (A), plasma cells (B) and lymphocytes (C) occupy this defect. There is no evidence of adrenal cellular regeneration or of fibroblastic activity. × 440.

FIG. 17. Area of necrosis is seen at A, small clusters of plasma cells in the interstitial tissues of zona fasciculata at B. × 440.

FIG. 18. Another view from the same case showing clusters of plasma cells (A) occupying defects in the cords in the zona fasciculata. × 440.



FIGS. 13-18

phagocytes and lymphocytes. There were also large phagocytes filled with typical crystalline hemosiderin confirmed by the Prussian blue reaction (Figs. 15 and 16). These areas were numerous in both adrenal glands but were not confluent.

V. *Case with Necrosis of Adrenal Cortex and with Evidence of Chronic Inflammation*

Case 13

A 47–1. A 57 year old white man was admitted to the hospital six months prior to his death for resection of the colon for adenocarcinoma. One ureter was cut accidentally at operation and a wound infection followed. One month after the initial operation he had daily chills and fever, lost weight and his appetite decreased. Jaundice and periods of disorientation characterized the remainder of his course. Autopsy revealed subacute hepatitis, acute left pyelonephritis, absence of the right ureter, ascites, and decubital ulcers. In addition microscopic examination showed advanced hemoglobinuric nephrosis, bilateral patchy areas of adrenal cortical necrosis and numerous foci of interstitial chronic inflammation, chiefly plasma cells (Figs. 17 and 18).

DISCUSSION

Our increased knowledge of the physiology of the adrenal cortex has given significance to lesions such as those described above, which, as Rich[6] has pointed out, were seen in their essential form by several investigators about the second decade of this century. One accepts the clinical importance of massive granulomatous, neoplastic, or fibrous replacement of cortical substance, while lesser degrees of injury to the adrenal cortex commonly escape recognition or discussion.

Simple necrosis, lacuna or pseudotubule formation, coagulative necrosis, with and without an inflammatory component are definite anatomic lesions. Their significance, however, must await clinical, biochemical, and postmortem correlations. This much has been established: the lesions are an integral part of the changes produced in monkeys in experimental diphtheria and malaria and are reported elsewhere.[3, 5]

I believe that the earliest recognizable *necrotic* lesion in the adrenal cortex consists first of loss of individual cell borders, solution of the cytoplasm, followed by pyknosis and extrusion of the nucleus. This usually involves a cluster of a few cells. A confluence of such necrotic foci leaves a space in the gland cortex, called a tubule by Rich[6] and Kinsman *et al.*,[2] and a lacuna or pseudotubule in this report. There is no leukocytic reaction to such necrotic foci in many cases, but it is present to a variable degree in other cases or different segments of the same gland. The examples cited above demonstrated the transition from simple necrosis to the usual cellular exudations of acute and chronic inflammation and prove that these foci are necrotic.

There are varied forms of necrosis encountered in the adrenal cortex. They are, however, associated commonly with the simple type of necrosis described above. One other type of necrosis is coagulative, in which each cell preserves its contour but loses all cellular detail; still another is a form of anemic necrosis bordered by leukocytes and, therefore, resembling an infarct. A further variant of this type of necrosis is the addition of hemorrhage into lacunae or pseudotubules, a view expressed by Kinsman *et al.* and by Rich, with which I concur. The hemor-

rhage appears to be produced by diapedesis and is dependent on a concomitant destruction of sinusoidal reticulum.

No examples of fibrous replacement are illustrated in this report. Extensive fibrosis, up to complete replacement of cortical substance, is a well known pathologic entity. On many occasions I have seen lesser degrees, down to fine interstitial scarring. Thus far, however, I have not seen an instance with evidence of chronic inflammation, active fibroblastic proliferation and dense collagenous stroma. However, in several cases that I examined there were present patches and linear streaks of fibrosis which by their distribution and configuration appeared to be sequels to focal necrosis.

There was no evidence that the severity of these changes is related to duration of illness in terms of number of days. We do not know the substance or substances responsible for adrenal cortical necrosis. Therefore, duration of illness is conceivably only one factor in the determination of the type and degree of adrenal cortical necrosis. The lesion may be reversible, as was demonstrated by Golden and Overman (loc. cit.) in experimental simian malaria. It also appeared that the lesion was prevented by the administration of lipoidal adrenal cortical extract.

An unsuccessful attempt was made to analyze human adrenal tissues to identify pre-necrotic lesions. Excessive cellular vacuolization, easily demonstrable as lipoidal accumulation, was not always seen in conjunction with these necrotic foci. In any event, lipoidal depletion and repletion of adrenal gland cortical cells is a labile process (in the rabbit, at least). Such cycles may be seen experimentally within a one-hour period. Furthermore, excessive cytoplasmic vacuolization, as shown in Figure 17, contained no stainable lipoid or bodies that were visible under crossed Nichol prisms.

It is reasonable to assume that such necrotic changes are repaired, usually short of fibrosis, when one considers the relative rarity with which one encounters even patchy adrenal fibrosis in subjects at autopsy. Greater changes, presumably beyond the capacity of cellular regeneration, probably result in variable degrees of fibrosis. I saw no evidence that complete fibrosis or atrophy of the adrenal cortex was preceded by lesions such as those described in this paper.

It must be emphasized that the lesions are patchy in distribution. Single sections may not reveal the lesions at all in a given case. Our most rewarding and least time-consuming method consisted of cutting each adrenal gland into 15 blocks (cross sections) and preparing 10 "step" sections from each block. It is my impression that for routine use, half the number of blocks and single sections would suffice to detect this type of necrosis in most cases.

CONCLUSIONS

1. Necrosis of the adrenal cortical cells exists as a pathologic entity and takes the form of simple, coagulative or anemic necrosis which is followed in time by mild leukocytic infiltration.

2. Hemorrhagic infiltration into the adrenal cortex is a variant of this type of necrosis.

3. This type of lesion usually exists in more than one form in the adrenals of a given case but has a patchy distribution.

4. The exact role of this lesion in the adrenal cortex of man remains to be demonstrated by combined biochemical and pathologic study.

REFERENCES

1. GOLDEN, A.: Morbid anatomy of typhus as seen in a recent Guatemalan epidemic; observations on the disease. Arch. Path., **39**: 226-231, 1945.
2. GOLDEN, A.: Adrenal gland lesions in experimental simian malaria and similar human lesions in varied diseases. Federation Proc., **7**: 271, 1948.
3. GOLDEN, A., AND OVERMAN, R. R.: Experimental cortical necrosis of the adrenal gland in the monkey in diphtheria and in malaria. Am. J. Clin. Path., **19**: 899-906, 1949.
4. KINSMAN, J. M., D'ALONZO, A. C., AND RUSSI, S. Fulminating meningococcic septicemia associated with adrenal lesions; analysis and discussion of seven cases. Arch. Int. Med., **78**: 139-169, 1946.
5. OVERMAN, R. R., BASS, N. E., DAVIS, A. K., AND GOLDEN, A.: The effect of lipo-adrenal extract on ionic balance in fatal simian malaria. Am. J. Clin. Path., **19**: 907-917, 1949.
6. RICH, A. R.: A peculiar type of adrenal cortical damage associated with acute infections, and its possible relation to circulatory collapse. Bull. Johns Hopkins Hosp., **74**: 1-15, 1944.

STERNAL MARROW STUDIES IN HODGKIN'S DISEASE

A Review of the Literature and a Report of Thirty-Five Cases*

LOUIS R. LIMARZI, M.D., and JEROME T. PAUL, M.D.

From the Department of Internal Medicine, University of Illinois College of Medicine, and Research and Educational Hospitals, Chicago, Illinois

It is generally agreed that Hodgkin's lymphogranulomatosis is not a disease of the lymph nodes exclusively but that the lesions may be widespread, following more closely the distribution of the reticulo-endothelial system than that of any other system. Although the ultimate diagnosis may in many cases rest upon the histologic examination of the affected lymph node, 12 per cent or more of patients with Hodgkin's disease do not show any lymphadenopathy for a long period of time.

Since Bunting's studies[7] the peripheral blood in Hodgkin's disease has been investigated by many authors.[23, 25, 57, 73, 78] Although some changes have been found, the diagnostic value of the blood picture has been disappointing.

REVIEW OF LITERATURE ON STERNAL MARROW IN HODGKIN'S DISEASE

Although the osseous lesions in lymphogranulomatosis were described by Benda[5] in 1904 and possibly by Hammer[26] in 1896, it is only in the last 22 years that any attempt has been made to evaluate the bone marrow findings in Hodgkin's disease obtained by sternal puncture.

Weiner and Kaznelson[96] in 1926 were the first to examine the sternal marrow obtained by aspiration in 2 cases of Hodgkin's disease but they did not find anything of particular interest. Tempka and Braun[85] examined the sternal marrow of 4 patients with untreated Hodgkin's disease and observed bizarre changes in the cytoplasm and nuclei of the granulocytic series which they believed indicated an atypical and hurried type of maturation.

Involvement of the bone marrow in Hodgkin's disease was studied by Steiner[80] (1943), who analyzed 14 cases, in 11 (79 per cent) of which microscopic examination of small samples of marrow revealed characteristic changes. In 38 of 62 bones selected at random, lymphogranulomatous foci were found. Although the vertebrae and bones of the pelvis seemed to be primarily attacked, the sternum was involved in 7 of the 11 cases (64 per cent) in which sternal marrow was examined. Steiner stated his belief that in practically every case of Hodgkin's disease lesions of the marrow would be found if enough bones could be thoroughly examined. Symmers[84] observed the specific lesion in the marrow in 7 of 14 cases of Hodgkin's disease. Bone marrow involvement was encountered in 19 of 39 cases reported by Turnbull.[88] Ziegler[103] stated that lesions of marrow are present in 30 to 40 per cent and Ewing[14] and Medlar[54] believed that involvement of marrow is the usual thing. Krumbhaar[38] reported a case of Hodgkin's disease in

* Aided by a grant from the Hematology Research Foundation, Chicago, Illinois.
Received for publication, May 24, 1949.

which necropsy revealed characteristic histologic changes in the bone marrow and the spleen, without detectable involvement of even a solitary lymph node Krumbhaar considered that the changes in the marrow may have been primary. With the wider use of X-ray it has been learned that the bones are not infrequently involved in Hodgkin's disease, although it is well known that patients with large lesions confined to the marrow may have negative roentgenograms. Steiner has stressed the fact that smaller lesions of the marrow are even harder to visualize and probably the great majority cannot be revealed by present roentgenographic technics and are unrecognized.

Markoff[53] examined the sternal marrow of 12 patients with Hodgkin's disease before and after roentgen therapy. An active neutrophilic reaction involving the stab neutrophils and neutrophilic myelocytes was observed in those cases with fever. The erythroid cells, megakaryocytes and reticulo-endothelial elements were normal even in patients with grave anemia. Eosinophilia of the marrow was sometimes augmented over that seen in the peripheral blood, especially in subjects with cutaneous manifestations of the disease. Plasma cellular reaction was observed in all the marrows. Irradiation caused a suppresssion of the normoblastic tissue and increased the number of neutrophils which later returned to normal. In the terminal phase of the disease and in cases of abdominal involvement the marrow was hypocellular and fatty. Markoff never observed lymphogranuloma cells in any of his cases, nor a histiocytic reaction, nor any other marrow elements that were of diagnostic value.

Nordenson[59] found in a patient with Hodgkin's disease (who did not receive any roentgen therapy) a shift of marrow cells to the left in the myeloid dispersion. In 3 other patients who received irradiation the bone marrow revealed a marked decrease in neutrophilic myelocytes with a corresponding elevation in the polymorphonuclear neutrophils. Myeloblasts were increased in all the marrows. Nordenson observed hypergranulation and alteration in the nucleo-cytoplasmic relationship. Plasma cells were common in all the marrows. Erythropoiesis was suppressed in the patient who received treatment and was normal in the others.

Rohr and Hegglin[70] in a patient with Hodgkin's disease found some cells with large nuclei in the bone marrow which they considered lymphogranuloma elements. In another study Rohr[71] noted myeloid hyperplasia with an increased number of immature granulocytes similar to that seen in all chronic infections.

Dameshek,[10] who examined the sternal marrow by means of biopsy in 6 cases of Hodgkin's disease, described an increase of the leukopoietic tissue with a shift to the left to include many myeloblasts and promyelocytes. Histiocytes were frequent; the megakaryocytes were normal in 5 patients and increased in a sixth. In one instance the bone marrow revealed a count of 36 per cent myeloblasts and promyelocytes.

Weil, Isch-Wall and Perles,[94] in a study of 20 cases of Hodgkin's disease, could not find anything that was characteristic in the bone marrow in this disease. They noted an increase in endothelial cells, some of which were distinguished with difficulty from megakaryocytes. Occasionally, they found an elevation in eosinophils, plasma cells and monocytoid cells. Weil and Perles[95] did not consider any finding in the bone marrow in Hodgkin's disease of diagnostic importance.

Mallarmé[52] studied the bone marrow in 7 patients with Hodgkin's disease and noted proliferation of eosinophils, monocytes and plasma cells. Reed-Sternberg cells were not encountered in any of the marrows even in a patient who showed bone marrow localization of the disease. Some of the monocytes were characterized by a vacuolated cytoplasm. Mallarme doubted that one can accurately recognize Reed-Sternberg cells in the bone marrow and stated that bone marrow eosinophilia does not constitute a typical finding.

Revol[68] studied the bone marrow and lymph nodes in 3 instances of lymphogranuloma. In 2 cases he found a definite neutrophilic reaction, a normal number of eosinophils and a reduction in the number of megakaryocytes. In a third case the bone marrow presented a neutrophilic myelocyte reaction and erythroblastic activity associated with a suppression of myeloblasts, hemocytoblasts and promyelocytes. There was no increase in megakaryocytes, and eosinophils were infrequent. Reed-Sternberg cells were not observed in any of the marrows. Revol agreed with Weil and Perles that sternal puncture in malignant lymphogranulomatosis does not carry the same diagnostic value that lymph node biopsy does.

Fieschi and Rettanni[19] reported on bone marrow studies in 8 patients in whom the diagnosis of Hodgkin's disease was made by histologic examination of an involved lymph node. In 4 of the patients the marrow was studied following irradiation. These investigators described the following 3 types of bone marrow patterns in this disease: (1) hyperplasia of the granulopoietic tissue with infrequent numbers of eosinophils: the maturation dispersion curve showed stimulation of the granulopoietic tissue (shift to the right), the so-called infective toxic syndrome; (2) hyperplasia of the erythroblastic tissue with relative erythroid immaturity; the erythroid maturation curve was that of inhibition. The authors referred to this phase of the disease as the syndrome of early anemia with a type of toxic inhibition of karyokinesis; (3) hypoplastic bone marrow, consisting mostly of lymphocytes, histiocytes and plasma cells, observed in preterminal cases and following roentgen-ray therapy. Besides these 3 types of bone marrow patterns, there were numerous varying intermediate possibilities. In the early stages of Hodgkin's disease, Fieschi and Rettanni did not observe any toxic involvement of granulopoiesis or any abnormalities of the nucleo-cytoplasmic relationship. These latter findings indicated that the process was advanced or that the patient had received irradiation. In some patients who had received large amounts of roentgen ray therapy, hypoplasia of the marrow was associated with frequent hemocytoblasts and atypical mitotic figures and anisocytosis of the myeloblasts and promyelocytes. The anemia of Hodgkin's disease was considered by these workers to be due to a hematoxic factor associated with the natural process of the disease or a maturation arrest of either the myeloid or erythroid tissue in which the spleen and reticulo-endothelial system take part. The latter mechanism interferes with the maturation of the erythropoietic and granulopoietic tissue and results in a hypercellular marrow with immaturity of myeloid and erythroid elements. The erythroid bone marrow pattern may resemble that seen in splenic anemia. These investigators never found the Reed-Sternberg lymphogranuloma cells in their material and they had little to say about the megakaryocytic tissue.

Introzzi[30] was of the opinion that in cases of malignant lymphogranuloma bone marrow studies are not of diagnostic value. Lymph node biopsy or splenic puncture in cases with definite splenomegaly are necessary in order for one to be certain of the diagnosis.

Barasciutti[1] made sternal aspiration studies in 6 patients with lymphogranulomatosis, 4 of whom did not receive any roentgen ray therapy. He observed a plasma cell reaction and normoblastic activity with frequent mitoses. There was no correlation between the number of eosinophils in the peripheral blood and that in the bone marrow. Barasciutti did not find Reed-Sternberg cells in the bone marrow in his patients with Hodgkin's disease.

Roversi and Tanturri[74] described hyperplasia of the granulopoietic tissue in Hodgkin's disease.

Rastelli[65] noted bone marrow eosinophilia with increased histiocytes and plasma cells and compared the bone marrow findings in Hodgkin's disease with that seen in chronic infection.

Parof, Fischgold, Abaza and Leui[61] did not observe any findings of diagnostic importance in the bone marrow in Hodgkin's disease although histologic studies of the lymph nodes and cutaneous lesions revealed Reed-Sternberg cells.

Stahel,[79] Tischendorf,[87] Leitner,[40] Velasco Montes,[90] Jagic and Klima,[33] Schulten,[75] Gasbarrini,[22] Thaddea[86] and Henning and Keilhack[29] described varying degrees of myeloid hyperplasia with eosinophilia and plasma cell reaction in their patients with Hodgkin's disease. Klima,[36] in addition to finding lymphogranuloma cells, described bone marrows with hypergranularization and abnormalities of the nuclei of the neutrophilic myelocytes and promyelocytes.

Parkes-Weber and Huber[60] reported the findings, in Hodgkin's disease, of large cells with a round nucleus which measured 7 microns in diameter, which were morphologically similar to cells seen in reticulosis.

Weerdt[93] noted marked bone marrow eosinophilia in Hodgkin's disease even in the absence of peripheral blood eosinophilia. Landolt[39] described a case of Hodgkin's disease with a leukemoid blood pattern and a white blood count of 122,000. The differential blood count showed 90 per cent eosinophils and the bone marrow revealed a marked eosinophilia. Major and Leger[51] described a similar case with a white count of 169,000, of which 99 per cent were eosinophils. The bone marrow presented a corresponding eosinophilia.

Klima[36] is one of the few investigators that claims to have observed lymphogranuloma cells, Lymphogranulomzellen, the precursors of Reed-Sternberg cells in the bone marrow in cases of Hodgkin's disease. Fleischacker and Klima[20] described the morphologic characteristics of these cells from the material obtained by lymph node puncture. The cells are described as large with an abundant lumpy and vacuolated cytoplasm that stains greenish blue. The nuclei are large and loose or vesicular with a rather fine reticulated chromatin which contains one or more nucleoli. These cells become enlarged, their nuclei lobulated, and they correspond to Reed-Sternberg cells. Because the development of these cells is not uniform one finds a pleomorphoric process with a predominance of lymphoblasts and lymphocytes in the lymph node whereas the myeloid cells and retic-

ulum cells are less frequent. Klima believes the lymphogranuloma cell is derived from the lymphoblast and not from a histiocytic development as considered by other investigators. In the bone marrow (sternal puncture) the number of lymphogranuloma cells are small and the immaturity of the lymphoblast is characteristic. Leitner[40] and others are not convinced that the cells described by Klima are specific elements, but believe that they are probably atypical plasma cells.

Kienle[35] found definite lymphogranuloma cells in the bone marrow in 5 of his 25 patients with Hodgkin's disease. The characteristic cells showed multiple sausage-like, subdivided nuclei which, with Giemsa's method, stained a bluish red color with large purple nucleoli. The cytoplasm was bluish, not granulated, small and frequently vacuolated. Aside from these cells, Kienle found other cells with a single large coarse nucleus and small nucleoli and abundant cytoplasm.

Ferrata and Storti[17] described and illustrated the Reed-Sternberg cells obtained from lymph node puncture in cases of Hodgkin's disease. The typical Reed-Sternberg cell as seen in these preparations was large and multinucleated; the nuclei varied from 2 to 5 in number, and as a rule were situated in the center of the cell. The nuclei appeared to overlap or were superimposed on one another and contained large nucleoli. Some nuclei were single and in such instances they were very large, multilobulated, and contained large nucleoli. The cytoplasm was abundant, slightly acidophilic in histologic sections and, on the other hand, rather basophilic in smear preparations. Reed-Sternberg cells in most nodes from Hodgkin's disease are sparse and scattered among other elements. Ferrata and Storti did not observe these cells in the sternal marrow from patients with Hodgkin's disease.

Leitner[40] reported on the bone marrow in 9 subjects with Hodgkin's disease who, in general, showed myeloid hyperplasia, eosinophilia and an increased number of plasma cells. The latter finding was most marked in progressive cases and in advanced cases with a slight anemia and hypoplasia of the erythroid tissue. Leitner described an instance of Hodgkin's disease in which the first sternal puncture revealed an erythroid hyperplasia and a slight increase in neutrophilic myelocytes with a hypercellular myeloid tissue. The second sternal puncture showed definite neutrophilic myelocytic hyperplasia, eosinophilia of 10 per cent with a peripheral blood eosinophilia of 5 per cent, frequent plasma cells and a few Klima lymphogranuloma cells which he considered to be atypical plasma cells. Puncture of the lymph node showed similar types of Klima lymphogranuloma cells as found in the bone marrow, lymphoblast-like cells, monocytoid cells but no Reed-Sternberg cells. Biopsy of a lymph node in this case revealed Reed-Sternberg cells, and confirmed the diagnosis of Hodgkin's disease. On the other hand, lymph node puncture material from the other patients all showed Reed-Sternberg cells.

Varadi[89] described the occurrence of Hodgkin's disease in a 39 year old man with minimal lymphadenopathy in whom sternal aspiration revealed the diagnosis by the finding of Reed-Sternberg cells. Roentgenographic studies of the bones were negative. The author admitted that very rarely the diagnosis of

Hodgkin's disease can be made by sternal puncture. Emile-Weil, in the discussion of this report, was not convinced that the cells noted by Varadi were Sternberg cells and remarked that the differentiation of these cells from megakaryocytes is often difficult.

Cordier, Barbier, Croizat and Vincent[9] reported a case of Hodgkin's disease with a leukemoid blood picture and a marked erythroblastic bone marrow (65 per cent erythroid cells) which they attributed to previous liver therapy. The leukocyte count in the blood was 10,000 with 44 per cent neutrophilic myelocytes.

Young and Osgood[102] found the marrow in 2 patients with Hodgkin's disease of no diagnostic value. No support was found for Medlar's view that there is a proliferation of the megakaryocytes in Hodgkin's disease.

Isaacs,[31] in 5 cases of Hodgkin's disease treated by roentgen ray and accompanied by anemia, noted a decrease in the number of nucleated forms of all types. The cells which remained appeared to mature in an orderly manner. There was no invasion of the marrow by foreign (neoplastic) cells as observed in cases of lymphosarcoma and lymphatic leukemia.

Vogel, Erf and Rosenthal[91] reported the occurrence of Hodgkin's disease in 5 adults, 3 of whom had intensive radiotherapy. The bone marrow differential counts revealed a slight "shift to the left", and in several cases an increase in eosinophils and reticulum cells. No Reed-Sternberg cells were found in the bone marrow in any of these patients. Vogel and Bassen[92] found nothing characteristic in the bone marrow in Hodgkin's disease in 3 children.

Morrison and Samwich[58] studied the sternal marrow in 22 patients with Hodgkin's disease. Most marrows showed only a slight erythroid activity. Of interest was the finding of an increase in reticulum cells, a moderate eosinophilia in the bone marrow or peripheral monocytosis. No Reed-Sternberg cells were found in the bone marrow in any instance. The myeloid-erythroid ratio was approximately 5.5 to 1.0. These investigators believed the bone marrow to be of great diagnostic aid in Hodgkin's disease.

Falconer and Leonard[15] examined the bone marrow in 14 patients with Hodgkin's disease and in 4 patients with lymphosarcoma and noted that a high leukocyte count with myeloid immaturity usually indicated skeletal involvement associated with either marrow metastasis or irritation of the bone marrow. The megakaryocytes were not increased. The sternal marrow in this group in some instances showed a leukemoid or myeloid reaction difficult to distinguish from the early myeloid reaction of myelogenous leukemia, although a lower myeloid-erythroid ratio was found more frequently in leukemia. In another series, Falconer and Leonard[16] studied the sternal marrow of 59 patients with Hodgkin's disease, in 11 of whom they found typical granulomatous lesions They noted an increased number of megakaryocytes. Mendell, Meranze and Meranze[55] found no characteristic changes in the marrow of 3 patients. Propp and Schwind[64] stated that the bone marrows in a patient with Hodgkin's disease and in another with mycosis fungoides were not of diagnostic value.

Limarzi,[44] in the study of several patients with Hodgkin's disease, noted a hypercellular bone marrow of a myeloid type with moderate increase in plasma

cells, reticulum elements and marked hyperplasia of the megakaryocytic element. Wolff and Limarzi[99, 100] studied the bone marrow in 2 women with Hodgkin's disease associated with pregnancy and found hyperplasia of the myeloid and megakaryocytic cells depending upon the stage and severity of the process. An increase in the number of plasma cells, histiocytes, reticulum cells and eosinophils was observed during some phase of the fatal disorder. (Pregnancy is usually not affected by, nor does it affect, this chronic slowly progressive disease and, therefore, may be treated in a conservative manner. Exceptions to this rule occur in advanced or fulminating Hodgkin's disease.)

Wolman[101] and Wintrobe[98] found the bone marrow picture in Hodgkin's disease to be variable and nonspecific. The lesions are focal rather than diffuse. There may be a shift to the left in the myeloid series, a slight monocytosis or moderate eosinophilia, and a relative reduction in nucleated red cells.

Piney and Paterson[63] commented that Hodgkin's disease affects lymphatic glands so much more obviously than it does marrow, that relatively little attention has been paid to the latter tissue. These investigators classified the cases in 4 groups: (1) those with normal bone marrow, (2) those with simple reactive hyperplasia of nonspecific type, (3) those with increased numbers of megakaryocytes and (4) those with obvious lymphogranulomatous infiltration. In the third group it is not, as a rule, possible to be certain that the giant cells are really Reed-Sternberg cells as there is no certain method of distinguishing them from megakaryocytes. The bone marrow findings may suggest Hodgkin's disease but such marrow changes are, by themselves, insufficient evidence. The fourth group which is very small, conforms to findings as reported by Rohr and Hegglin. Piney and Paterson, in summary, stated that the majority of patients with Hodgkin's disease show a progressive anemia of the hypoplastic type, which only becomes severe in the terminal stages and then the bone marrow shows the corresponding changes described with this type of anemia.

Scott[76] studied the bone marrow from 8 instances of typical Hodgkin's disease. The puncture findings varied, depending upon the stage of the disease; in none were Reed-Sternberg cells or Lymphogranulomzellen of Klima found. In 3 patients there were various degrees of shift to the left of granulopoiesis, and in 2 a moderate increase in megakaryocytes. Scott was of the opinion that the changes found on sternal puncture in Hodgkin's disease are as variable and as nonspecific as the changes in the peripheral blood.

Sundberg[81] stated that the lesion of Hodgkin's disease is extremely difficult to demonstrate on the basis of an examination of sternal marrow.

Campion and Diggs[8] observed a hypercellular marrow in 7 persons and a hypoplastic marrow in another who had received large amounts of roentgen ray therapy. The marrow was usually cellular and the myeloid-erythroid ratio, essentially normal. There was a slight myeloid immaturity. The eosinophils, mononuclears, histiocytes and sometimes the lymphocytes were slightly increased. Atypical cells which were morphologically on the borderline between reticulum cells, large mononuclear and promyelocytes were demonstrable in all smears. These large atypical cells were characterized by folded, indented or lobulated

nuclei and an early nuclear structure. Plasma cells were noted and megakaryocytes, mostly of the mature variety, were present in increased numbers.

Paula e Silva,[62] in 3 cases of Hodgkin's disease, found Reed-Sternberg cells and a hypercellular marrow with a quantitative elevation in plasma cells, monocytes, monoblasts and the type of cells found by Tempka and Braum. Transitional forms were observed between the "gigantic cells" (Reed-Sternberg) and those of the reticulo-endothelial system. No abnormal mitotic figures were noted. The anemia of Hodgkin's disease is explained on the basis of a toxic inhibitory factor on the bone marrow. Bastistoni and Perazzini[3] studied the sternal marrow, spleen and lymph nodes by means of aspiration in 7 patients with Hodgkin's disease. The bone marrow showed an increase in plasma cells and occasional Reed-Sternberg cells. The authors contended that of these three procedures, lymph node puncture was the easiest and safest method of diagnosis of Hodgkin's disease. Bernard[6] believed that it is very difficult to make the diagnosis of Hodgkin's disease by study of the sternal marrow. He noted hyperplasia of the myeloid tissue and frequently eosinophilia of the bone marrow.

MATERIAL AND OBSERVATIONS

This report comprises the hematologic and clinical observations made on a group of 35 patients with Hodgkin's disease admitted to the University of Illinois Research Hospitals. In addition, 10 patients with lymphosarcoma, 5 with reticulum cell sarcoma and 2 with mycosis fungoides were studied. The diagnosis was established in all cases by biopsy or autopsy. All patients had complete hematologic examinations in addition to the study of the aspirated sternal marrow.

There were 20 males and 15 females. All were white except for one Negro and one oriental. The youngest patient was 10 years of age and the oldest 75 years. Twenty-four patients were in the age group of 10 to 40 years and 11 were in the 40 to 70 year old group. An attempt to ascertain the duration of the disease was made at the time of the initial examination. It was determined that in 21 of the patients the disease had been present for two years or less. In 7 patients the duration was believed to be two to three years; in one instance the duration was five years and in another, eight years. Lymphadenopathy was found in 34 of the 35 persons. The cervical glands were involved in 32 (94 percent), the axillary in 18 (53 per cent) and the inguinal in 16 (47 per cent). The spleen was palpable in 16 (46 per cent) persons and the liver was palpably enlarged in 13. X-ray examination of the chest revealed evidence of enlarged hilar glands or lung infiltration in 24 patients. Cutaneous involvement was noted in 3 instances.

The method of sternal aspiration and preparation of marrow specimens and the apparatus have been described in detail elsewhere.[4, 41, 43, 44, 45, 49, 50] To state it briefly: Under aseptic technic and procaine anesthesia the sternum (midline) is punctured in the second or third interspace, preferably the former site, with a specially devised 16-gauge needle. This needle*[42] is a modification of the one

* This needle, called The University of Illinois Sternal Needle, is manufactured by V. Mueller & Co., 408 South Honore Street, Chicago 12, Illinois.

devised by Klima and Rosegger.[37] The needle is forced into the bone perpendicular to the sternum until a sudden "give" indicates that the marrow cavity has been reached. The stylet is then removed. With a tight fitting, dry, 5 or 10 ml. syringe, exactly 1 ml. of marrow fluid is aspirated and immediately placed in a paraffin-lined tube containing a minute amount of heparin. The tube is gently inverted several times to insure the anticoagulant effect of the heparin. Usually, if a successful sample of bone marrow has been obtained, a more or less dense suspension of small tissue particles will be visible. The 1 ml. of heparinized material consisting of peripheral blood (sinusoidal), hemopoietic marrow and fat is pipetted into a Wintrobe hematocrit tube and centrifuged for approximately five minutes at about 2000 r.p.m. Centrifugation separates the following layers, reading from above down in the tube: (1) fat, (2) plasma, (3) myeloid-erythroid cells (this layer also contains the megakaryocytes), and (4) erythrocytes from bone marrow sinuses. The height of each of the layers is recorded. The fat and most of the plasma are removed separately and discarded. The myeloid-erythroid layer, together with a small amount of plasma, is pipetted off separately, transferred to a paraffin-lined watch glass, mixed, and from this material films and cell counts are made. The above method has been followed by the author and his associates in more than 4150 examinations of the bone marrow and peripheral blood made at the same time. A small drop of marrow fluid is transferred to a new and perfectly clean microslide and with an 18 x 22 mm. rectangular cover glass a margin-free film is made. The film is stained with Wright's stain or May-Grünwald-Giemsa dyes. A control film that has been made from a drop of non-heparinized marrow fluid is also prepared in the same way.

When the stained films of the bone marrow were studied, the ratio of myeloid to erythroid cells, the dispersion of the nucleated red cell series, the dispersion of the myeloid cells series, and the number of megakaryocytes were recorded. Megakaryocytes were classified as immature, mature and degenerated types. In recording the erythropoietic elements, the terms pronormoblast, basophilic, polychromatophilic, and orthochromatic normoblast were used, in order of increasing maturity. Similarly, for the granulopoietic series, myeloblast, leukoblast, promyelocyte, myelocyte, metamyelocyte, stab forms and polymorphonuclear neutrophil were used. Histologic studies of the bone marrow were made from bone marrow particles of the marrow obtained by sternal aspiration or biopsy material obtained by a small trephine.

DISCUSSION OF RESULTS AND ANALYSIS

Peripheral blood studies (Table 1) in the group of Hodgkin's disease revealed the following findings. The hemoglobin in 16 patients was found to vary from 6 to 10 grams, in 3 patients it was below 5 grams, and in the remaining 16 between 11 and 15 grams. In 27 patients the erythrocytes were above 3,000,000 per cu. mm. The anemia was normocytic in type in most of the cases.

The leukocyte count was below 5000 per cu. mm. in 8 patients, above 15,000 in 8 patients, and between 5000 and 15,000 in 19. The differential pattern was not characteristic. Two patients had significant eosinophilia (17 and 27 per cent).

TABLE 1

PERIPHERAL BLOOD FINDINGS IN NORMAL PERSONS AND IN PATIENTS WITH HODGKIN'S DISEASE, LYMPHOSARCOMA, RETICULOSARCOMA AND MYCOSIS FUNGOIDES

	HODGKIN'S DISEASE (35 PATIENTS)		NORMAL (10 PERSONS)	LYMPHOSARCOMA (10 PATIENTS)		RETICULOSARCOMA (5 PATIENTS)		MYCOSIS FUNGOIDES (2 PATIENTS)
	Average	Range		Average	Range	Average	Range	
Hemoglobin (Gm./100 ml. blood)	10.2	4.5–15.5	14.9	12.7	8.8–14.9	14.3	13–16	13.8
Erythrocytes (Mil.)	3.80	1.31–5.33	5.0	4.62	3.65–5.06	4.80	4.36–5.60	4.44
Leukocytes (Thous.)	10.0	2.0–28.6	7.45	9.44	2.0–23.6	8.18	4.80–11.2	15.0
Platelets (Thous.)	Normal to increase		250.0 350.0	Normal		Normal to increase		Normal
Hematocrit (erythrocytes), %	33.4	13–50	44.6	41.4	27–50	43.6	41–49	42.5
Hematocrit (leukocytes), %	1.0	0.5–3.0	0.5	1.0	0.5–4.0	0.6	0.5–1.0	1.0
Sedimentation rate (uncorrected; mm. in 1 hr.)	46.0	7–81	10.5	26.0	3–57	24.0	12–35	28.0
Sedimentation rate (corrected; mm. in 1 hr.)	23.0	3–42	7.3	18.5	0–28	23.0	8–38	26.0
Mean corpuscular volume (cu. mm.)	87.0	71–105	88.5	88.5	74–101	91.0	87–98	95.0
Mean corpuscular hemoglobin (micromicrograms)	26.0	20–35	29.5	27.3	24–30	29.8	25–33	30.5
Mean corpuscular hemoglobin conc., %	29.0	23–36	33.5	30.5	24–33	32.2	31–33	32.0
Reticulocyte count, %	1.6	0.1–27	0.3	0.9	0.5–3.0	0.9	0.8–1.0	0.6
Icterus index (units)	5.8	2–15	6.5	6.75	5–10	7.0	5–10	6.0
Differential Blood Smear								
Neutrophilic metamyelocytes, %	0.3	0–4	0	0		0.2	0.2–1	0
Stab forms (neutrophils), %	12.6	0–41	5.1	13.4	0–38	14.2	9–26	4.5
Polymorphonuclear neutrophils, %	56.3	11–96	54.5	55.5	35–82	57.2	41–75	72.0
Lymphocytes, %	18.6	1–59	29.5	19.7	8–45	16.8	4–33	11.0
Monocytes, %	8.5	0–17	8.9	7.4	3–24	8.4	5–14	8.0
Eosinophils, %	3.0	0–27	1.4	3.3	1–9	3.0	0–9	4.0
Basophils, %	0.7	0–3	0.6	0.7	0–2	0.2	0–1	0.5

In 5 instances the eosinophils numbered 3 to 10 per cent of the differential count. A tendency for a slight to moderate monocytosis was observed in many of the cases studied. Nineteen patients demonstrated a monocyte percentage of 7 to

TABLE 2

BONE MARROW FINDINGS IN NORMAL PERSONS AND IN PATIENTS WITH HODGKIN'S DISEASE, LYMPHOSARCOMA, RETICULOSARCOMA AND MYCOSIS FUNGOIDES

	HODGKIN'S DISEASE		NORMAL PERSONS	LYMPHOSARCOMA		RETICULOSARCOMA		MYCOSIS FUNGOIDES
	Average	Range		Average	Range	Average	Range	
Number of subjects	35		10	10		5		2
Quantitative Values								
Myeloid-erythroid volume, %	15.6	1.0–50.0	6.8	12.1	3–45	5.8	1–10	6.7
Fat volume, %	1.9	0–17.0	3.2	4.3	0–10	8.0	2–20	1.0
Megakaryocytes (per 18 mm. square)	Moderate to marked increase		52.0	Moderate increase		Moderate increase		Normal
Qualitative Findings								
Myeloid series (%)								
Myeloblasts	0.3	0–3.0	0.5	0	0	0.4	0–2.0	0.5
Leukoblasts, neutrophilic	11.8	0–39.0	2.0	12.7	6.0–23.0	13.6	6.0–26.0	6.0
Promyelocytes, neutrophilic	5.1	0–20.0	5.0	6.9	2.0–21.0	8.0	1.0–14.0	11.5
Myelocytes, neutrophilic	12.2	1.0–28.0	10.5	16.9	5.0–58.0	11.8	6.0–18.0	11.5
Metamyelocytes	44.3	2.0–71.0	51.0	41.2	20.0–67.0	39.6	33.0–50.0	36.0
Stab neutrophils	3.4	0–30.0	2.0	0	0	0	0	0
Polymorphonuclear neutrophils	17.2	3.0–76.0	24.0	13.8	5.0–28.0	18.2	5.0–40.0	21.0
Eosinophils (all types)	5.5	1.0–25.0	4.5	8.2	0–17.0	8.0	4.0–12.0	13.0
Basophils (all types)	0.2	0–3.0	0.5	0.3	0–3.0	0.4	0–2.0	0.5
Erythroid series (%)								
Pronormoblasts	2.8	0–17.0	2.6	2.8	0–6.0	1.8	1.0–5.0	1.5
Basophilic normoblasts	7.4	1.0–34.0	13.2	6.6	2–17.0	7.0	3.0–12.0	4.0
Polychromatic normoblasts	89.4	49.0–100	78.3	90.6	80.0–96.0	88.8	81.0–96.0	94.5
Orthochromatic normoblasts	0.4	0–8.0	5.9	0	0	2.4	0–12.0	0
Myeloid-erythroid per cent and ratio	70:30 (2.3:1)	30:70–97:3 (0.4:1–32:1)	73:27–35:65 (2.71:1.0)	73:27 (2.7:1)	55:45–82:18 (1.2:1–4.5:1)	75:25 (3:1)	52:48–90:10 (1.1:1–9:1)	79:21 (3.7:1)

17. The number of polymorphonuclear neutrophils was inconstant but a definite shift to the left was noted. Twenty-three of the 35 patients had a percentage of stab neutrophils above the usually accepted normal value of 5 per cent. In 9 instances the number of stab neutrophils exceeded 25 per cent of the differential count. Neutrophilic metamyelocytes were observed in 6 patients. The blood platelets were normal in number in 19 patients, decreased in 5 and increased in 11 patients.

Quantitative Changes in Bone Marrow

The bone marrow was hyperplastic in 25 of the 35 patients with Hodgkin's disease. The volume of nucleated bone marrow cells reached values as high as 50 per cent with an average reading for all cases (Table 2) of 15.6 per cent. This is more than twice the normal volumetric value. The fat volume (volumetric reading) averaged 1.9 per cent with the highest reading of 17 per cent in a 70 year old man. In 10 cases of Hodgkin's disease the hypoplasia of the bone marrow elements was indicated by a reduced volumetric reading with values as low as 1.0 per cent. The hypocellular marrows were seen in terminal cases of Hodgkin's disease, some of which had received frequent courses of roentgen-ray therapy. In several cases hypoplasia of marrow elements was due to Hodgkin's invasion of the bone marrow. These findings were confirmed at autopsy. Although not reported as a part of this paper, we have observed several cases of Hodgkin's disease with practically complete aplasia of the bone marrow following nitrogen mustard therapy. These were terminal cases of Hodgkin's disease which had become refractory to roentgen-ray therapy. At autopsy the marrow was composed mostly of fat with aplasia of the hemopoietic elements.

Qualitative Changes in Bone Marrow

A hypercellular marrow was found in most of the patients with Hodgkin's disease who had a moderate to marked degree of granulopoietic immaturity. Immaturity was never carried to the stage of myeloblastic involvement. Myeloblasts averaged 0.3 per cent with a high value of 3.0 per cent in one case. In most of the marrows myeloblasts were infrequent or were not encountered in the differential count of the myeloid series. The greatest increase occurred in the leukoblasts which, in some cases, comprised as much as 39 per cent of the granulopoietic series. This is a marked increase when compared with the normal value of 2.0 per cent. Although individual cases showed marked elevations in the number of neutrophilic metamyelocytes, myelocytes and promyelocytes, in general the average value for all the cases of Hodgkin's disease studied are within the normal range. Some patients presented marked eosinophilia of the bone marrow and others very pronounced polymorphonuclear neutrophilia, although the latter finding in some cases can be attributed to admixture with peripheral blood. Most patients with Hodgkin's disease present a normal maturation dispersion curve except for hypercellularity of myeloid cells. Apparently normal (homoplastic) granulopoiesis is accelerated and the insignificant myeloid immaturity observed in some of these marrows does not involve the mechanism of hetero-

plastic myelopoiesis. Blood patterns with a leukemoid reaction or with moderate to marked increase in stab neutrophils were often associated with a hyperplastic bone marrow pattern with variable degrees of shift to immaturity, simulating the bone marrow pattern in chronic myeloid leukemia. The bone marrow in these cases of Hodgkin's disease showed, besides toxic changes in the granulocytic series, an increase in the number of reticulum cells and plasma cells similar to that seen in many toxic-infectious states. Severe toxic changes in the granulopoietic tissues were indicated by moderate to marked degrees of "toxic" granules which may fill the cell and obscure the nucleus. These toxic granules were observed in many of the granulopoietic elements, even in phases of mitotic division. These toxic changes of the granulopoietic tissue are similar to those described by the authors[67] in the bone marrow in cases of infectious mononucleosis. Marked degenerative changes of the nucleus or cytoplasm with the formation of vacuoles, abnormalities in the size, shape, and staining affinity of the nucleus and anisocytosis of the granulocytic cells in the various phases of maturation were also observed. Only rarely were disparity and disorganized maturation between the nucleus and cytoplasm, atypical mitotic figures and complex nuclear configurations found. In general, mitosis appeared normal even in those marrows with a severe toxic condition of the granulopoietic tissue. Irradiated cases of Hodgkin's disease without hypoplasia or aplasia of the bone marrow, nitrogen mustard-treated cases, and terminal cases which had become refractory to treatment frequently showed the more marked toxic changes in the myeloid tissue. It was interesting to observe very marked hyperplasia of the myeloid tissue with moderate to marked left shift in the maturity dispersion without any morphologic sign of toxic granulopoiesis in patients with Hodgkin's disease who had received a few roentgen-ray treatments or a single course of nitrogen mustard therapy. In most cases, eosinophilia of the bone marrow could be correlated with a peripheral blood eosinophilia, while in other cases, moderate to marked eosinophilia of the marrow was associated with a normal number of eosinophils in the blood. There was no correlation between the clinical findings (lymphadenopathy, splenomegaly and cutaneous lesions) and the eosinophilia of blood or bone marrow. Toxic changes were not observed in the eosinophils either in the blood or bone marrow, even in the presence of a severe toxic involvement of the neutrophilic tissue in the bone marrow.

A cell type first described by Hargraves and his associates[28] and termed "Tart" cell was seen in most of the bone marrow smears from cases of Hodgkin's disease. This cell has also been observed in the bone marrow from patients suffering from pulmonary infections, metastatic carcinoma, and a number of other benign and malignant disorders. As originally described by Hargraves and his associates, the cell is usually a histiocyte or a "monocytoid reticulo-endothelial" cell that contains a second characteristic nucleus usually placed in the Hof of the primary nucleus. This second nucleus nearly always stains reddish purple with Wright's stain in contrast to the dark purple nucleus of the histiocyte. This secondary nucleus shows a sharp nuclear membrane and the chromatin is definitely visible in thick structural arrangement with relatively sharp differentiation between

the chromatin and parachromatin. In our cases of Hodgkin's disease the phenomenon was observed more frequently in the mature neutrophilic polymorphonuclear leukocyte in the bone marrow than in any other type of marrow cell. As noted by Hargraves, we also have never observed "Tart" cells in the peripheral blood of patients with Hodgkin's disease. There was no correlation between the number of these cells and the stage of the disease, although they were more frequently seen in hypercellular marrows.

Erythropoiesis

In most of the bone marrows from patients with Hodgkin's disease who had not received therapy for the disease, the erythroid tissue was normal. In several marrows, groups or clusters of normoblastic elements were noted which were probably indicative of an erythroid stimulation similar to that seen in chronic infections. The anemia in these cases was frequently normocytic in type. Pronounced erythroid hyperplasia with marked normoblastic immaturity was observed in several cases of Hodgkin's disease associated with a hemolytic component and in a few cases with a microcytic hypochromic anemia in the peripheral blood. Some cases with either a microcytic hypochromic anemia or a simple microcytic anemia in the peripheral blood revealed a myeloid hyperplasia with a moderate left shift in the myeloid dispersion maturation curve. In spite of the microcytic hypochromic anemia in the peripheral blood, erythropoiesis appeared to be suppressed. This is contrary to the normoblastic hyperplasia observed in cases of iron-deficiency anemia with a microcytic hypochromic anemia in the peripheral blood associated with chronic hemorrhagic anemia. In none of the cases of Hodgkin's disease with a hypochromic anemia was there any evidence of chronic loss of blood. Megaloblastic erythropoiesis was not observed in any of the bone marrows even when the anemia was macrocytic in type in the peripheral blood. Karyorrhexis of the normoblastic nuclei was seen in practically all the bone marrows, more marked in long standing cases and in those receiving roentgen-ray or nitrogen mustard therapy.

Some patients with terminal Hodgkin's disease and those receiving large doses and frequent courses of roentgen-ray or nitrogen mustard therapy occasionally showed moderate to marked degree of erythroid hypoplasia, but aplasia of this tissue was never seen in our series. Disparity and disorganized maturation between the nucleus and cytoplasm, bizarre nuclear configuration, or multipolar mitosis of the erythroid tissue as reported by some investigators were not observed in our material.

Megakaryocytopoiesis

Moderate to extreme megakaryocytic hyperplasia was one of the most constant bone marrow findings in patients with Hodgkin's disease. In most of the marrows the morphology and maturation dispersion of the megakaryocytes did not vary from normal. In patients with generalized lymphadenopathy and in those with moderate to marked splenomegaly, megakaryocytic hyperplasia was usually associated with myeloid hyperplasia with moderate im-

maturity, reticulum hyperplasia, plasma cellular reaction and frequent karyorrhexis of the normoblastic nuclei. Immaturity of the megakaryocytes was a frequent finding in these marrows. Many of the immature megakaryocytes were morphologically normal; others showed atypical and less frequently bizarre nuclear configuration. Mitotic figures were observed in the early and late stages of megakaryocytopoiesis, but this was an infrequent finding. Many of the early megakaryocytes (immature) were of the lymphoid or hyaline type with a basophilic cytoplasm lacking granules. The nucleus was often polymorphic and coarse without nucleoli. Normally when the megakaryocytic nucleus is polymorphic the cytoplasm contains azurophilic granulations and is not as basophilic and does not have peculiar streaks of hyaloplasm and irregular areas of spongioplasm as in the pathologic types. Some marrows showed an increase in the number of both degenerating megakaryocytes and megakaryocytes mechanically damaged or ruptured, with extrusion and breaking up of the large multilobulated nucleus. In the former state, after completing its platelet-forming function, the cytoplasm of the megakaryocytes becomes greatly depleted, disintegrates and the nucleus is represented by a polymorphous mass which is dark-staining, pyknotic and stripped of almost all its cytoplasm. The megakaryocytes ruptured or damaged mechanically during the preparation of the smear, lack the characteristics described for degenerating megakaryocytes.

Some investigators consider that the giant cells (Reed-Sternberg cells) in Hodgkin's disease are megakaryocytes. Symmers[84] described deformed and degenerated megakaryocytes in sections of the bone marrow together with identically deformed and degenerated megakaryocytes in the lymph nodes, the spleen and the liver. Symmers regarded them as emboli from the marrow, and Medlar[51] interpreted them as cells metastasizing from a primary malignant tumor of the marrow or megakaryocytoblastoma. As Symmers has stressed, there have not been any comprehensive studies of film preparations of marrow cells in Hodgkin's disease, all of the comparatively few studies having been made of fixed tissues in which cell details are poorly differentiated. Recently Rebuck,[66] by the use of imprint preparations of the bone marrow, spleen and lymph nodes, studied, among other things, the distinguishing morphologic characteristics of the megakaryocytes and the giant cells (Reed-Sternberg cells).

Downey,[12] Barta,[2] Jurgen and Graupner,[34] Willi,[97] Ferrata,[18] Di Guglielmo,[11] Seeliger,[77] Frank,[21] Rohr,[69] Rosenthal,[72] Limarzi[48] and many others have described the origin, formation, and morphology of the megakaryocytes in human bone marrow and in experimental animals under normal and pathologic conditions; therefore, only those references pertinent to the present material will be mentioned.

Briefly, the megakaryocytes may be divided into immature, mature and degenerative forms. The mature cells comprise those conforming to the classic description and also the small young megakaryocytes frequently seen in the chronic hemorrhagic anemias with azurophilic granules appearing in the perinuclear zone. Immature megakaryocytes include megakaryoblasts and promegakaryocytes. The young megakaryoblasts begin their development from the myelo-

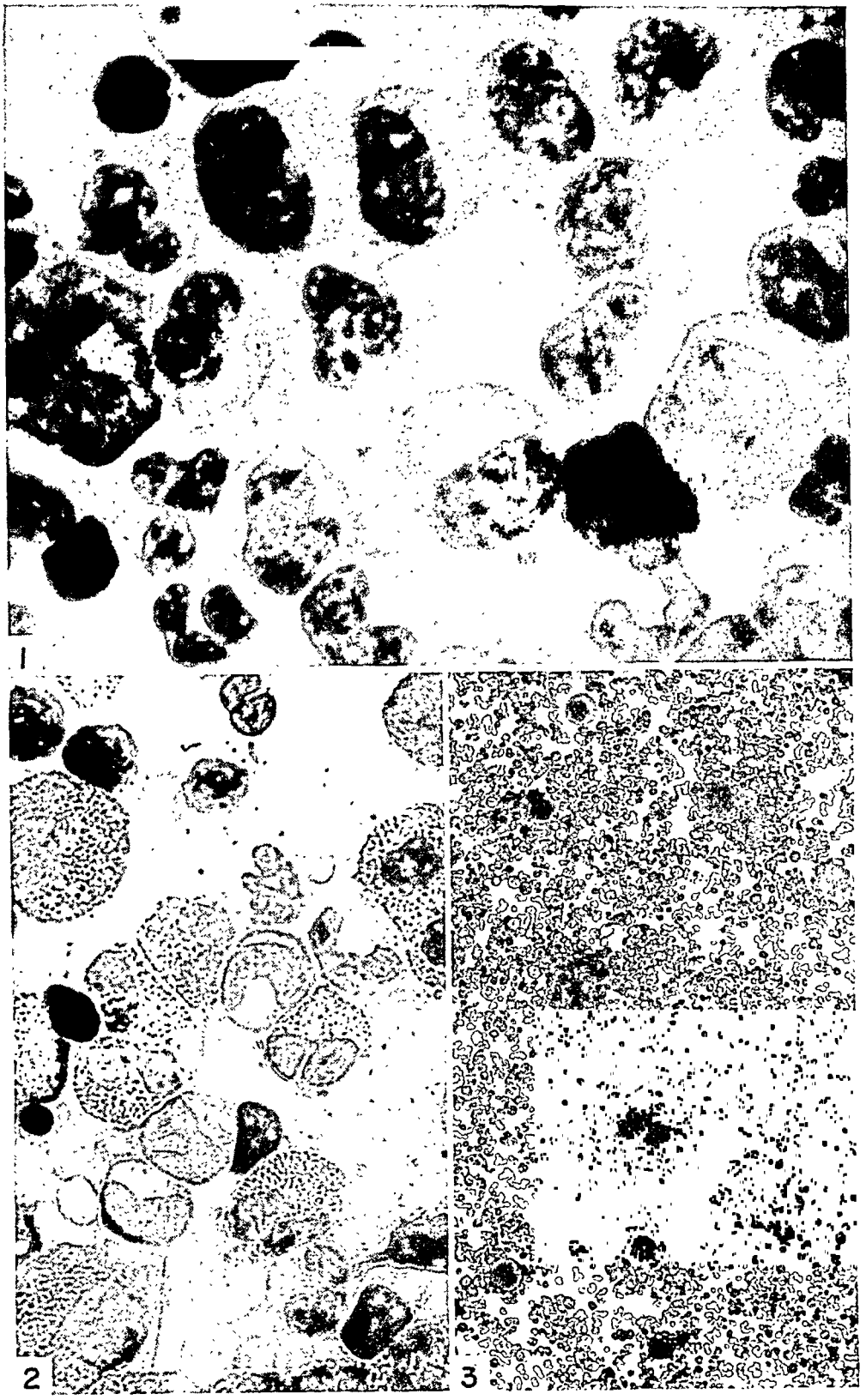
blast (hemocytoblast) by an increase in thickness and density of the chromatin strands and by change of their karyoplasm from slightly basophilic to faintly acidophilic, while at the same time the cytoplasm becomes more basophilic. There are no granules and no cytoplasmic zones at this stage. Promegakaryocytes of the next stage of development show beginning azurophilic granulation of the cytoplasm and maturing of the nucleus, but show no indication of platelet formation. Coincident with increase in the size of the cell, the nucleus divides mitotically or amitotically and a coarse net of chromatin is formed within it ("lymphoid megakaryocyte"). Azurophilic granules accumulate at first in the perinuclear zone, later filling the entire cytoplasm. Normally the breaking up of the cytoplasm into platelets occurs only in cells which have reached a certain stage of growth and development and proceeds rapidly once it begins. It takes place in various ways, but usually either by a process of budding or detachment of portions of the cytoplasm (Wright's figure), or by dissolution of the cytoplasm in the bone marrow. All or most of the cytoplasm of the megakaryocyte is given off to the blood and the nucleus degenerates as previously described. The more or less naked nucleus is often carried by the blood stream to the lungs where it lodges in the capillaries.

In Hodgkin's disease the productivity of the megakaryocytic and myeloid tissue is accelerated and is associated with hyperplasia of these tissues, but without any marked increase in leukocytes or platelets in the peripheral blood. It is true that polymorphonuclear leukocytosis, myeloid immaturity and elevated platelet counts are observed in some cases of Hodgkin's disease during some phase of the disease. However, more frequently the bone marrow shows moderate to extreme myeloid and megakaryocytic hyperplasia without any corresponding changes in the peripheral blood. Abnormalities of the granulopoietic tissue were infrequent while pathologic changes of the megakaryocytes were observed in many of the bone marrows in Hodgkin's disease. These abnormalities were almost always observed in immature megakaryocytes of the lymphoid type and were associated with hyperplasia of the megakaryocytes. The lymphoid megakaryocytes were observed in different stages of development and degeneration. The smaller ones were round, only slightly larger than the myeloblasts. They had a single nucleus that was comparatively large and a very coarse chromatin network with a narrow rim of deeply basophilic cytoplasm and contained no granules. The nucleus in many of these cells was almost "hyaline" in character. Other cells of this size contained several nuclei or a complex nucleus similar to the mature form but with a coarser and darker chromatin structure. Large pathologic lymphoid megakaryocytes, some the size of very large mature megakaryocytes (35–40 microns in diameter), were observed with a single, rarely a multilobulated, nucleus or multiple nuclei, which stained intensely purple, often dark and muddy due to the intense staining of both chromatin and parachromatin.

FIG. 1. Bone marrow (Hodgkin's disease) showing hypercellularity and myeloid cells in various phases of development. × 1100.

FIG. 2. Bone marrow eosinophilia in a case of Hodgkin's disease. Note the eosinophils in various stages of development, one being in mitotic division. × 1000.

FIG. 3. Megakaryocytic hyperplasia in the marrow in Hodgkin's disease. × 400.



FIGS. 1–3

The nuclei in some cells were still "hyaline" or "lymphoid" in type and the cytoplasm was abundant and deeply basophilic and contained no granules. Some of the larger cells contained a large nucleus with one or more smaller nuclear masses. In forms of lymphoid megakaryocytes, apparently degenerating, nuclear fragmentation was observed with the formation of nuclear buds and nuclear masses. Further pathologic and functional alterations of these cells were seen in the formation of small and larger pseudopodal masses which became isolated from the main cell mass. Some were small and foamy and had irregular outlines, others were deeply basophilic and had smooth surfaces. Some of these detached elongated cytoplasmic masses contained a few fine azurophilic granules, but there was no morphologic indication of normal platelet formation. In a few cases of Hodgkin's disease these abnormal cytoplasmic structures were seen in the peripheral blood. On account of the appearance of the nucleus and cytoplasm of the pathologic lymphoid megakaryocyte, one would be inclined to believe the cells had originated in the reticulum, although direct evidence for such an opinion is lacking from a morphologic point of view. These pathologic types of megakaryocytes are not to be confused with degenerative forms of functionally normal mature megakaryocytes recognized by chromatolysis and pyknosis of the nucleus and by loss of distinctness and brilliancy in cytoplasmic granules. Although naked nuclei are morphologic evidence of degeneration of the megakaryocytes, one cannot definitely distinguish those resulting from mechanical damage from those due to normal degenerative changes.

Bone Marrow Reticular Cells

The reticular tissue in the bone marrow in Hodgkin's disease is made up of undifferentiated and histiocytic types of reticular cells. The undifferentiated form is a large cell with a relatively large amount of cytoplasm.[82] The nucleus is round or oval in shape with a delicate chromatin pattern. The fine nuclear chromatin stains light reddish purple and is distinctly demarcated from the pale pink-staining parachromatin. Nucleoli when present are variable in size and may be partially obscured by overlying chromatin. The nuclear membrane is fine but distinct, in contrast to the actual boundaries of the cytoplasm of the undifferentiated reticular cell which are often not clearly visible. The cytoplasm is frequently damaged and appears to be watery, and sometimes vacuoles and azurophilic granules also can be found. Histiocytic reticular cells, the other

FIG. 4. "Tart" cell in the marrow from a case of Hodgkin's disease. Note the second nucleus in the cytoplasm of the polymorphonuclear neutrophil, the nuclear lobes of which are pressed to one side of the cell. × 1440.

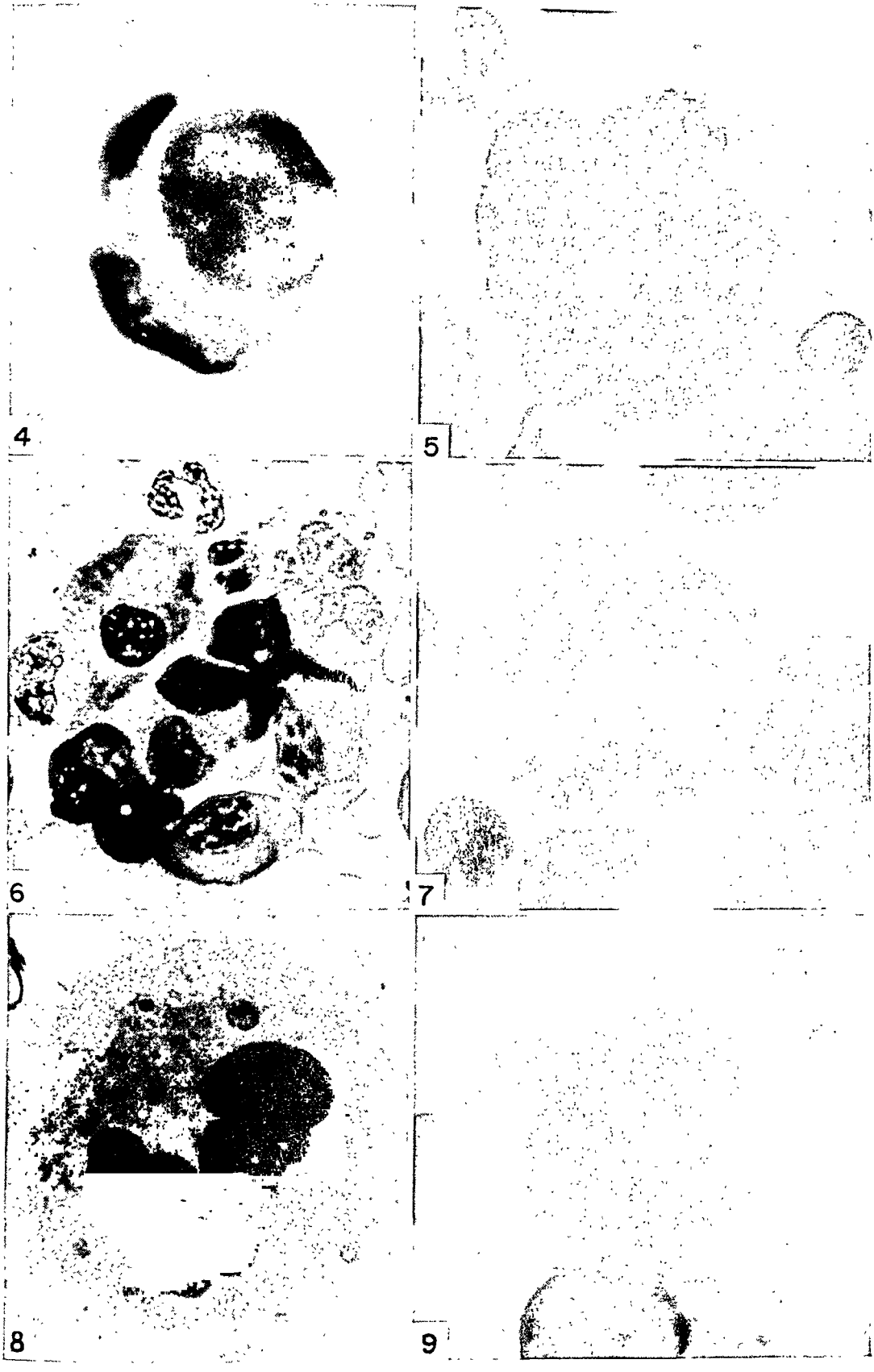
FIG. 5. Lymphoid megakaryocyte showing bizarre nuclear configuration and lack of azurophilic granules in the cytoplasm from bone marrow in Hodgkin's disease. × 1000.

FIG. 6. Bone marrow in Hodgkin's disease showing a group of plasma cells. × 1000.

FIG. 7. Lymphoid megakaryocyte showing bizarre nuclear configuration and lack of azurophilic granules in the cytoplasm from bone marrow in Hodgkin's disease. × 1000.

FIG. 8. Normal mature megakaryocytes with multilobulated nuclei and azurophil granules in the cytoplasm from the bone marrow in a case of Hodgkin's disease. × 1200.

FIG. 9. Promegakaryocyte showing sharp differences between inner dark granular zone and outer lighter and more homogeneous zone. Note the atypical platelet formation from cells of this type in the bone marrow from case of Hodgkin's disease. × 1200.



FIGS. 4–9

type of reticular cells seen in the bone marrow in Hodgkin's disease, are very similar to the undifferentiated type, have distinct cytoplasmic boundaries and definite aggregation of the nuclear chromatin. Many of these cells appear to be potential phagocytes (histiocytes), as evidenced by the phagocytosed material contained in the cytoplasm of cells. Reticular cells of one type or another were observed in most of the bone marrows in Hodgkin's disease, although their relative number varied from case to case, without any relationship to the stage or duration of the disease. Patients who received several courses of roentgen-ray therapy, frequent blood transfusions or had an associated hemolytic anemia, usually presented hyperplasia of the histiocytic reticular cells with moderate to marked phagocytic characteristics. Mitosis in reticular cells was a rare finding.

There was no morphologic evidence in our bone marrow material for the transformation of the undifferentiated reticular cell to any type of hemopoietic elements or to the Reed-Sternberg cell. Rebuck, in a comprehensive study on the structure of the giant cells in the blood-forming organs, illustrated and described in detail the development of the atypical reticulum cells of the Reed-Sternberg type in imprint preparations of lymph nodes in Hodgkin's disease and compared them with aspirated marrow megakaryocytes in the different stages of development and degeneration. Since Reed-Sternberg cells of Hodgkin's disease were not observed in our bone marrow material obtained by sternal aspiration, they will not be discussed in detail in this paper. For cytologic details and excellent photomicrographs of the developmental stages of Reed-Sternberg cells in lymph node preparations, the reader is referred to the article by Rebuck.[66]

Bone Marrow Plasma Cells

A plasma cellular reaction was observed in most of the bone marrows in the cases of Hodgkin's disease. The plasma cells were singularly scattered throughout the bone marrow or found in groups. Although the ordinary mononuclear plasma cells were the type frequently present in the marrow of cases of Hodgkin's disease, multinucleated types with 2 to 5 discrete nuclei were occasionally observed. Morphologically they did not vary from those seen in normal bone marrow or the plasma cellular reaction seen in certain subacute and chronic inflammatory conditions, agranulocytosis and aplastic anemia.[56]

Plasma cells as seen in bone marrow smears are not quite similar to those seen in stained tissue sections. In dry-fixed preparations the nuclei are round or oval and the chromatin granules are arranged in coarse clumps but present chromatin-parachromatin distinctions. The nuclei are frequently eccentrically placed with

FIG. 10. Reticulum cell hyperplasia in the bone marrow in Hodgkin's disease. × 1000.

FIG. 11. Two large pathologic immature megakaryocytes with multilobulated nuclei, lack of azurophilic granules in the cytoplasm. From the bone marrow of a patient with Hodgkin's disease. × 1200.

FIG. 12. An immature megakaryocyte showing lobulation of nucleus and pseudoplatelet formation. Note the lymphoid megakaryocyte in the phase of mitosis. Bone marrow in Hodgkin's disease. × 1200.

FIG. 13. Two small immature megakaryocytes showing lobulation of the nuclei, lack of azurophilic granules in the cytoplasm and pseudoplatelet formation in the bone marrow of Hodgkin's disease. × 1000.



FIGS. 10–13

a central light area and abundant basophilic cytoplasm which occasionally is vacuolated. The spokelike arrangement of the nuclear chromatin ("Radkern") seen in tissue sections is not observed in bone marrow smear preparations. Atypical forms of mononuclear or multinuclear plasma cells were not observed in our cases of Hodgkin's disease.

Lymphosarcoma and Reticulum-Cell Sarcoma

Besides the normal controls, blood and bone marrow were studied in cases of lymphosarcoma, reticulum cell sarcoma and mycosis fungoides, in view of the close relationship of these conditions with Hodgkin's disease.

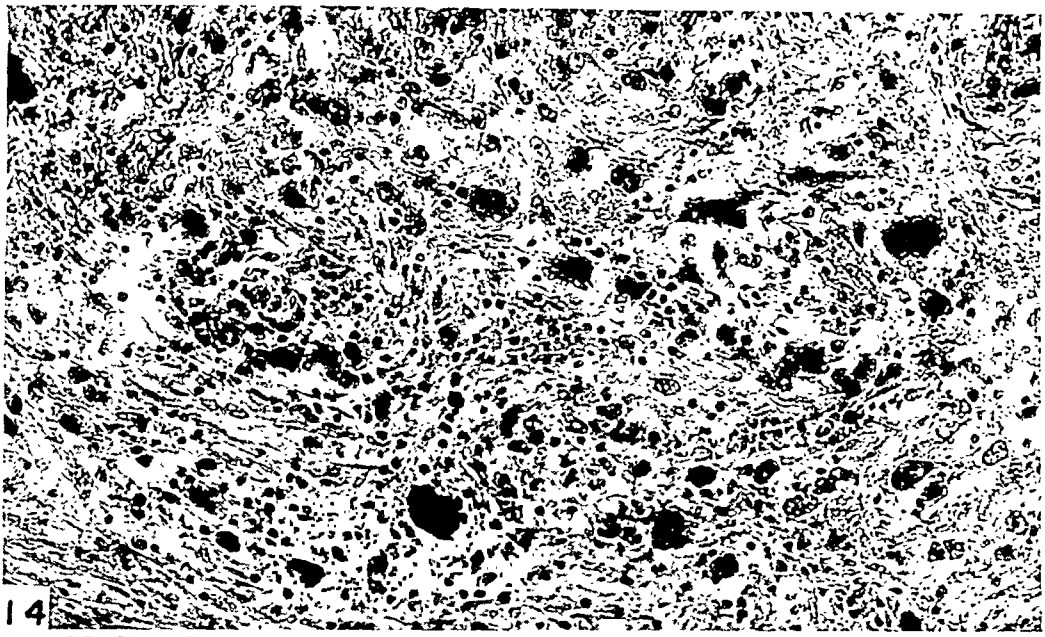
The group with lymphosarcoma included 9 males and one female patient, all white. The youngest patient was eight years of age and the oldest 71. The duration of the disease was believed to be six months or less in 5 cases. The series included one case of nine years' duration and another of five years' duration. Lymphadenopathy was found in 9 of the 10 patients. The cervical glands were involved in 8, the axillary in 6 and the inguinal in 7. The spleen was palpable in 3 patients. X-ray examination of the chest revealed evidence of enlarged hilar glands in 6 patients.

The hemoglobin ranged from 11 to 15 grams in 9 of the patients and the erythrocyte level was found to be above 4,000,000 per cu.mm. in 8 patients (Table 1). The anemia was normocytic in type. The leukocyte count was below 5000 per cu.mm. in 2 patients, above 15,000 in one patient and between 5000 and 15,000 in 7 patients. The differential blood pattern revealed an increase in eosinophils, monocytes and stab neutrophils in 5 of the subjects. The blood platelets were normal in number in 8 instances.

The series included 5 patients with reticulum cell sarcoma. Four of the patients were 50 years of age or older. Lymphadenopathy was found in all. The cervical glands were involved in 5 patients, the axillary in 3 and the inguinal in 4. The spleen was palpable in only one of the 5. X-ray evidence of enlarged hilar glands was present in one patient. Peripheral blood studies were essentially within normal limits except for a moderate increase in the number of stab neutrophils and an eosinophilia of 9 per cent in one person.

According to these findings, the bone marrow in lymphosarcoma and reticulum cell sarcoma, except for the hyperplasia, did not show any abnormalities. In a few cases not included in this report, the authors have observed a leukemic blood picture occurring either spontaneously or induced by roentgen-ray therapy. Early spontaneous lymphosarcoma-cell leukemia shows infiltration of the bone marrow which may be the first sign of the disease. The transition of lymphosarcomatosis to leukemia has been observed by a number of investigators.[27] With the development of the leukemic phase severe anemia, neutropenia and thrombocytopenia occur.

In mycosis fungoides neither the peripheral blood nor the bone marrow had any diagnostic significance. Attention has been called to the fact that mycosis fungoides is not a disease entity but a conglomerate of cutaneous changes varying from incidental eruptions to tumor-like growths and tumors.[83]



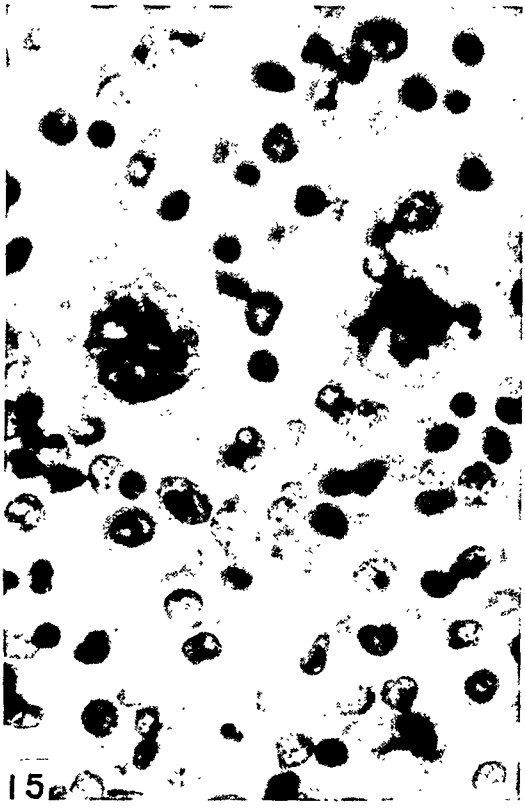




FIG. 14. Histologic section (sternum) of bone marrow from a case of Hodgkin's disease, taken at autopsy. Note the Reed-Sternberg cells in various phases of development. × 400.

FIG. 15. Histologic section (sternal marrow particles) from bone marrow in Hodgkin's disease showing two mature megakaryocytes surrounded by many cells of the granulocytic series. × 800.

FIG. 16. Oil immersion magnification of Figure 14. Note the large Reed-Sternberg cell in the center of the field surrounded by others in various phases of development. × 1000.

DISCUSSION

The two theories concerning the nature of Hodgkin's disease are (1) that it is a process of myeloidization of hyperplastic lymphoid tissues, possibly due to a virus infection, and (2) that it is a primary malignant tumor of lymph nodes or of the bone marrow. Pathologists recognize several types described by such terms as lymphoblastoma, reticulum cell sarcoma, lymphosarcoma, Hodgkin's disease and giant follicular lymphoma. The fact that the pathologic picture may sometimes pass from one type to another suggests that the disease is fundamentally unitary rather than made up of separate entities.

Although many investigators favor the neoplastic nature of Hodgkin's disease, its clinical course and behavior resemble more closely certain chronic infections than they do other malignant tumors. Jackson and Parker[32] consider that the histopathologic features, clinical course and type of fever are all indicative of the infectious nature of the process.

In most of our cases, *anemia*, if present, was either normocytic or hypochromic, rarely simple microcytic, microcytic-hypochromic or macrocytic in type. Severe anemia was usually associated with hypoplasia of the erythroid tissue but aplasia of this tissue was not recorded in the 35 cases included in this report. Normal, active erythropoiesis was the rule in these cases. Marked erythroid hyperplasia with a shift in erythrogenic tissue toward immaturity was seen in several patients with Hodgkin's disease who had abdominal involvement (splenomegaly) and no other clinical findings. These patients in addition showed hyperplasia of megakaryocytic elements, moderate to marked myeloid hyperplasia and granulopoietic immaturity. In these cases the anemia was normocytic in type with a tendency toward leukopenia and a normal platelet count. Splenectomy was performed in two of these patients, and in one was followed by death several months after the operation, and by marked clinical improvement without any change in the blood and bone marrow in the other. It is interesting that in this case the characteristic fever of Hodgkin's disease (Pel-Ebstein) persisted after splenomegaly and only disappeared following a course of nitrogen mustard. In our patients with Hodgkin's disease and marked splenomegaly but no other clinical finding, the bone marrow changes resembled those described by Fieschi[19] and Limarzi and his associates[46, 47] in cases of Banti's syndrome and other splenomegalic states. It is important that the diagnosis of Hodgkin's disease be excluded before splenectomy. In rare instances there is a hemolytic anemia of considerable severity associated with reticulocytosis, polymorphonuclear leukocytosis and thrombocytosis. The bone marrow showed erythroid hyperplasia with marked immaturity and macrocytosis in the peripheral blood. In one case (not included in this report) there was no alteration in the fragility of the red blood cells and spherocytes were not demonstrated. Splenectomy was performed without any effect on the hemolytic syndrome. Roentgen-ray therapy, nitrogen mustard and blood transfusions similarly had no effect on the hemolytic anemia. This patient died at home and although no autopsy was obtained, histologic sections of the spleen showed the typical Hodgkin's granulomatous lesions.

The *cause of the anemia* in Hodgkin's disease is not entirely clear. It has been

attributed to involvement of the bone marrow, toxic suppression of the erythroid tissue and to agents used in the treatment of the disease (X-ray and nitrogen mustard). It is generally agreed that patients with Hodgkin's disease treated by roentgen ray as well as those treated with nitrogen mustard, may develop an aplasia of all the bone marrow elements with associated marked anemia, severe leukopenia (agranulocytosis) and thrombocytopenia. Fortunately we did not observe aplasia in our cases. In fact, in several of our patients following treatment with roentgen ray and nitrogen mustard, there was an initial hyperplasia of all the bone marrow elements followed by a gradual or prompt return to a normal bone marrow pattern. The peripheral blood occasionally revealed a moderate leukopenia and thrombocytopenia, but more often the blood remained unchanged. The hyperplasia in the bone marrow concerned chiefly the granulopoietic tissue with a moderate to marked left shift to the leukoblastic stage without myeloblastic involvement. The megakaryocytic elements revealed an increase in the number of immature forms (normal and pathologic types of lymphoid megakaryocytes) and "naked" megakaryocytic nuclei (degenerated forms). Although the exact explanation of the anemia is yet to be determined, the involvement of bone marrow is not the entire answer in view of our findings of an active normal, and often hyperplastic, erythroid tissue. A factor which suppresses the final maturation and delivery of the red cells to the peripheral blood seems to be present. It is of interest that patients with Hodgkin's disease with a microcytic and hypochromic anemia and a normoblastic bone marrow do not respond to iron therapy. Also, the pronormoblastic type of bone marrow seen in a few cases of Hodgkin's disease did not resemble the megaloblastic type of erythropoiesis seen in pernicious anemia, as reported by a number of investigators. Neither the peripheral blood nor bone marrow simulated the picture seen in severe, relapsing pernicious anemia. In Hodgkin's disease one observes macrocytic red cells which are uniform and round, instead of showing great variation in size and shape. The macropolycytes of pernicious anemia are not found in the peripheral blood in Hodgkin's disease and the bone marrow shows none of the peculiar disturbances of myeloid tissue and megakaryocytes seen in the former disease. Patients with Hodgkin's disease showing marked myeloid-erythroid and megakaryocytic hyperplasia, increase in reticular cells (histiocytic hyperplasia) and macrocytic anemia in the peripheral blood presented findings simulating those of liver cirrhosis. The macrocytic anemia is best explained on the basis of involvement of the liver plus the toxic mechanism associated with Hodgkin's disease. The frequent finding of toxic changes in the granulocytes in the peripheral blood and granulopoiesis in the bone marrow in the presence of an intact erythroid tissue seems to speak for a chronic toxic process, which is associated with a disease of infectious nature, as the cause of anemia.

The *leukocyte count* in most of our cases of Hodgkin's disease ranged from 5000 to 15,000 per cu.mm. The highest white count of 28,600 per cu.mm., as was true in most cases with polymorphonuclear neutrophilic leukocytosis, was seen in advanced stages of the disease. The bone marrow in many of these cases showed myeloid hyperplasia with varying degree of left shift but more often a

right shift in the myeloid maturity dispersion. The toxic state of the granulopoietic tissue in the bone marrow was reflected in the peripheral blood by a moderate to marked toxic neutrophilia with a moderate shift to the left in the neutrophilic blood pattern. Myeloid immaturity in the peripheral blood was seen in several cases. In one case, not included in this report, with generalized involvement of the bone marrow (proven at autopsy), a leukocyte count of approximately 50,000 was associated with severe myeloid immaturity including many leukoblasts and neutrophilic promyelocytes, myelocytes and metamyelocytes, but with infrequent myeloblasts. Along with the myeloid immaturity the peripheral blood also contained nucleated red blood cells, Howell-Jolly and Cabot ring bodies and basophilic stippling of the erythrocytes. There was a moderate reticulocytosis; the icterus index was normal. Sternal puncture revealed a markedly hyperplastic bone marrow with immaturity of the erythroid and myeloid elements, but without myeloblastic involvement. Karyorrhexis of the normoblastic nuclei was frequent. The megakaryocytes were increased in number with frequent immature forms that showed abnormalities in cytoplasmic fragmentation. The latter observation was reflected in the peripheral blood by the finding of bizarre types of platelets. No Reed-Sternberg cells were found in the bone marrow smears, yet at autopsy the bone marrow showed the typical granulomatous lesions of Hodgkin's disease with numerous Reed-Sternberg cells in different stages of development. There was nothing constant or characteristic concerning the cases associated with either blood or bone marrow eosinophilia. Monocytosis of the blood was observed in patients who received roentgen-ray therapy, but was never associated with a monocytosis of the bone marrow. A lymphopenia in the peripheral blood and bone marrow was frequently observed. The basophils were unaffected by the disease.

The *blood platelets* were normal or increased in number but never definitely reduced on examination of the blood smear. There was no correlation between the number of blood platelets and the megakaryocytic hyperplasia in the bone marrow. In most of the cases the morphology of the platelet was normal while in a few cases abnormalities of the platelets were associated with an increase in the number of pathologic lymphoid megakaryocytes in the bone marrow. Here again there is no strict correlation between the number of pathologic lymphoid megakaryocytes in the marrow and the number and morphologic characteristics of the pathologic forms of platelets in the peripheral blood.

Although cells occasionally confused with Reed-Sternberg cells include multinucleated plasma cells, foreign body giant cells, Langhans' giant cells and tumor giant cells, it is the *megakaryocytes* that have caused the most confusion from a histopathologic point of view. Symmers and Medlar take the position that the giant cells in Hodgkin's disease are megakaryocytes. Histologically, the lesions are often miliary in type, composed of the typical granulomatous tissue which replaces the normal marrow. The characteristic lesions are confined for the most part, if not exclusively, to the sites of the red bone marrow—the ribs, sternum, spine, clavicle, skull, or the bones about the shoulder or pelvic girdle. Apparently the lesions are productive of great activity on the part of the hemopoietic

elements at the periphery of the process. This marked hemopoietic activity is evidenced by the hypercellularity observed on sternal aspiration (volumetric studies) which involves all the marrow elements. The hyperplastic megakaryocytic tissue and the miliary lesions with Reed-Sternberg cells may be situated side by side. Thus it is apparent that in a specimen of marrow removed for biopsy, unless a certain degree of fibrosis is present, great difficulties will be encountered in distinguishing megakaryocytes from Reed-Sternberg cells.

Megakaryocytes closely resemble Reed-Sternberg cells. There is no doubt, as Jackson and Parker and others have noted, that given a single cell (section), it is almost impossible to determine which of the two it is, although megakaryocytes do not have prominent nucleoli and their nuclei, since multilobed, are always single. The cytologic features of Reed-Sternberg cells developing from atypical reticulum cells and the formation of megakaryocytes from myeloblastic tissue as observed in film preparations of lymph nodes and bone marrow, offer differentiating criteria that cannot be obtained from histologic material. The cytologic development of the Reed-Sternberg cell from the small atypical reticulum cell to the large typical cell with its giant, bizarre, and polymorphous nuclei has been described by a number of investigators in histologic and imprint preparations of the lymph node. In general the nucleus of the characteristic giant cells in Hodgkin's disease is large in proportion to the size of the cell which may be single or multiple. In the mononucleated cell the nucleus is usually round, although bean-shaped and irregular indented nuclei are common. Reed[67] noted that when the nuclei are multiple they are arranged peripherally in the cell or heaped in the center with as many as eight to ten nuclei in a cell. Rebuck called attention to the large acidophilic nucleoli, thin nuclear membrane, relatively few particles of chromatin and scanty acidophilic cytoplasm in some of the atypical reticulum cells from imprints of nodes in Hodgkin's disease. The large round or rod-shaped acidophilic nucleoli which are characteristically prominent in the simple form of giant lobulated Reed-Sternberg cells and their relatively moderate chromatin content are in sharp contrast to the nuclear characteristics of the early and late developmental states of the mature megakaryocytes. Furthermore, the presence of azurophilic granules and of budding, detached or degenerated granular cytoplasm of the megakaryocyte is never observed in the cytoplasm of the Reed-Sternberg cells. Degenerated forms of Reed-Sternberg cells, which are a constant feature of Hodgkin's disease, may be confused with degenerated forms of megakaryocytes. This cytologic differentiation becomes more difficult in the hyperplastic marrow of cases of Hodgkin's disease when the number of degenerated megakaryocytes is increased and the degenerated forms of Reed-Sternberg cells which are a constant feature throughout the course of Hodgkin's disease become more frequent following roentgen-ray and nitrogen mustard therapy. In general it should be noted that the fine reticular chromatin network of the Reed-Sternberg cells retains its chromatin-parachromatin distinction until and beyond the stage of actual beginning cellular degeneration. The nucleoli retain their deep, clear, basophilic staining properties and the cytoplasm remains unchanged.

In contrast, in the late stage of the mature megakaryocyte, the pyknotic nucleus may assume a pachychromatic pattern. The nucleoli are absent or masked and the clumping of the azurophilic granules in small masses in the cytoplasm becomes more apparent when pseudopodial platelet formation is present. These cytologic features distinguish this cell from the degenerated Reed-Sternberg cell.

It should be stressed that the pathologic types of megakaryocytes observed in some marrows in Hodgkin's disease are not peculiar to this disorder. They have been observed in a number of other diseases of the hemopoietic tissues, and Downey and Nordland[13] described similar types of atypical megakaryocytic development in a case of myeloid megakaryocytic hepatosplenomegaly. As pointed out by other investigators, there is in Hodgkin's disease, an increase in bone marrow megakaryocytes which bears no correlation to the platelet count. Some of the pathologic megakaryocytes (atypical lymphoid forms) and early mature types show degenerative changes of the nucleus including pyknosis and karyolysis, and may be incapable of functioning in their platelet-forming capacity.[24] This pathologic type of platelet formation is reflected in the peripheral blood by the common occurrence of large fragments of megakaryocytes and the great variation in the size of platelets.

The productivity of the normal bone marrow is enormous and, under pathologic conditions, may be accelerated or retarded. In Hodgkin's disease there is marked hyperplasia of megakaryocytic and myeloid tissue without corresponding reflection in the peripheral blood. It is probable that many of the maturing megakaryocytes and granulocytes normally die *in situ* and are never delivered to the peripheral blood. At any rate, the mechanism of proliferation and maturation of the granulocytic and most megakaryocytic elements is intact, but apparently there is an interference with release of mature granulocytes and platelets to the peripheral blood. Perhaps the toxic process associated with Hodgkin's disease acts on the bone marrow to produce myeloid and megakaryocytic hyperplasia; or a suppression of their delivery (release mechanism), beyond the normal limit, to the peripheral blood which results in a myeloid and megakaryocytic hyperplasia. It is interesting to note that other benign toxic states may similarly affect the myeloid and megakaryocytic elements in the bone marrow.[46]

It is established that many of the pleomorphic cellular elements of the granulomatous lesion in the lymph nodes in Hodgkin's disease are seen in increased numbers in the bone marrow. According to Symmers, Hodgkin's disease is a process of myeloidization of hyperplastic lymphoid tissue, possibly due to a virus infection. Symmers takes the position that the giant cells in Hodgkin's disease are megakaryocytes since in one and the same case of Hodgkin's disease he observed deformed and degenerated megakaryocytes in the lymph nodes, spleen, and liver. On the other hand, there are many authorities who disagree with the view that the giant cells in Hodgkin's disease are megakaryocytes. Only Symmers and Medlar contend that they are megakaryocytes. Symmers regards them as emboli from the marrow and Medlar interprets them as cells metastasizing from a primary malignant tumor of the marrow, a megakaryocytoblastoma.

Our detailed cytologic studies on the bone marrow megakaryocytes in cases of

Hodgkin's disease, and those of Rebuck on the Reed-Sternberg cells from imprints of lymph nodes, are additional evidence for the opinion that the giant cells in Hodgkin's granuloma are not similar or related to the platelet-forming cell, the megakaryocyte. The evidence at hand definitely indicates that the cellular marrow elements removed by sternal aspiration in most cases of Hodgkin's disease are not obtained from the pleomorphic lesion characteristic of Hodgkin's granuloma. Such is the case in spite of the fact that the focal granulomatous lesions are often scattered throughout the bone marrow. This is supported by the failure to find the typical Hodgkin's lesions in histologic studies of sternal bone marrow particles obtained from the aspirated material or in biopsy material taken with a small trephine. This failure to observe the specific tissue in sternal aspirated material is due to several reasons, as stressed by Steiner: (1) not every sternum is involved in Hodgkin's disease; (2) the scattered specific focal lesions usually occupy such a small proportion of the marrow cavity as compared with the non-involved bone marrow itself that, on the basis of chance, they may not be aspirated by the sternal needle; (3) the specific lesions in the bone marrow tend to have much fibrous tissue making their aspiration more difficult. It cannot be too strongly stressed that unless specific tissue (in section) is observed, the diagnosis of Hodgkin's disease should not be made. Individual marrow cells or groups of cells as seen in sternal aspirated smears are not sufficient evidence on which to make the diagnosis of Hodgkin's disease. The marrow smear itself does not reveal any pattern diagnostic of the disease. In view of the fact that the disease carries such a poor prognosis, the diagnosis must depend on the pleomorphic picture of the histologic section as a whole, preferably from an involved lymph node.

SUMMARY

This paper reviews the literature of bone marrow findings in Hodgkin's disease, and reports the clinical, hematologic and bone marrow studies in a series of 35 patients with Hodgkin's disease.

1. The most constant finding in the bone marrow in Hodgkin's disease is myeloid and megakaryocytic hyperplasia. The morphologic pattern of the granulopoietic tissue is that seen in chronic toxic states. The panhyperplasia of the bone marrow simulates that of Banti's disease (splenic anemia).

2. A pathologic type of megakaryocytopoiesis involving the lymphoid megakaryocytes is observed in some cases of Hodgkin's disease. These produce atypical forms of platelets.

3. Reed-Sternberg cells are not seen in aspirated sternal marrow or in the histologic sections of bone marrow particles from such material. Evidence is presented to show that the giant cells of Hodgkin's granuloma are neither similar to nor related to bone marrow megakaryocytes.

4. The finding of increased numbers of normal or atypical plasma cells, eosinophils and reticular cells is neither a constant nor a specific pattern of the bone marrow in Hodgkin's disease. Eosinophilia of the bone marrow cannot be correlated with the peripheral blood eosinophilia.

5. The accurate diagnosis of the disease is established only by the finding of

the specific pleomorphic lesion of Hodgkin's granuloma. This is best observed in the histologic section of an involved lymph node. The finding of any one cell or group of cells in sternal aspirated bone marrow smears is insufficient evidence for the diagnosis of Hodgkin's disease.

REFERENCES

1. Barasciutti, A.: Sulla scarsa utilita della sternopunctura come mezzo diagnostico nel granuloma maligno. Diagnostica e Tecnica Labor., **8**: 481–485, 1937.
2. Barta, I.: Über Bau und Funktion der Megakaryocyten. Folia haemat., **47**: 168–179, 1932.
3. Bastistoni, L., and Perazzini, F.: La diagnosi citotogica des granuloma maligno per puntura midollare, splenica e ghiandolare. Haematologica, **30**: 89–112, 1947.
4. Bedinger, P. L., and Limarzi, L. R.: Clinical interpretation of sternal puncture. Illinois M. J., **94**: 351–356, 1948.
5. Benda, C.: Zur Histologie der pseudoleukämischen Geschwülste. Centralbl. f. allg. Path. u. path. Anat., **15**: 542–543, 1904.
6. Bernard, J.: Maladies du Sang et des Organes Hematopoietiques. France: Edition Medicales Flammarion, 1948.
7. Bunting, C. H.: Blood platelets and megalokaryocytes in Hodgkin's disease. Bull. Johns Hopkins Hosp., **22**: 114–116, 1911.
8. Campion, W. M., and Diggs, L. W.: Bone marrow studies in Hodgkin's disease. Cleveland Clinic Quarterly, **15**: 36–41, 1948.
9. Cordier, V., Barbier, J., Croziat, P., and Vincent, D.: Granulomatose maligne a type myeloide et diagnostique due myélogramme. Interet Clinique Soc. Med. Hop. Lyon, 24 XI, 36 in Lyon Med., **1**: 22–26, 1937.
10. Dameshek, W.: Biopsy of sternal bone marrow; its value in the study of diseases of blood forming organs. Am. J. M. Sc., **190**: 617–640, 1935.
11. Di Guglielmo, G.: Sul sistema delle celluli giganti midollari. Haematologica, **6**: 156–195, 1925.
12. Downey, H.: The origin of blood platelets. Folia Haemat., **15**: 25–58, 1913.
13. Downey, H., and Nordland, M.: Hematologic and histologic study of a case of myeloid megakaryocytic hepato-splenomegaly. Folia Haemat., **52**: 1–39, 1939.
14. Ewing, J.: Neoplastic Diseases. Ed. 4, Philadelphia: W. B. Saunders Company, 1940.
15. Falconer, E. H., and Leonard, M. E.: The value of sternal marrow aspiration as a method of bone marrow biopsy. Ann. Int. Med., **15**: 446–458, 1941.
16. Falconer, E. H., and Leonard, M. E.: Skeletal lesions in Hodgkin's disease. Ann. Int. Med., **29**: 1115-1131, 1948.
17. Ferrata, A., and Stori, E.: Le malattie del sangue. Milano: Societá Editrice Libraria, 1946.
18. Ferrata, A.: Le Emopatie. Milan: Soc. Edit. Lib., 1918.
19. Fieschi, A.: Semeiologia del Midollo Osseo in Ferrata, A.: Biblioteca Haematologica. Vol. 6. Pavia: Typografia gia Cooperativa, 1938.
20. Fleischacker, H., and Klima, R.: Zellbilder von Lymphknotenpunktaten und ihre diagnostische Verwertbarkeit. München. med. Wchnschr., **84**: 661–664, 1937.
21. Frank, E.: Die hamorrhagischen Diathesen in Schittenhelm. Handbuch der Krankheiten des Blutes und der blutbildenden Organe. Vol. 2. Berlin: Julius Springer, pp. 289–472, 1925.
22. Gasbarrini, A.: Linfogranuloma maligno acuto. La Clinica, **5**: 515–526, 1939.
23. Goldman, L. B.: Hodgkin's disease: Analysis of 212 cases. J.A.M.A., **114**: 1611–1616, 1940.
24. Gunn, F. D.: Reactions of the bone marrow in experimentally induced thrombocytosis. Arch. Path., **12**: 153–179, 1931.
25. Haden, R. L., and Burns, J. T.: Hodgkin's disease, review of 47 cases. Cleveland Clinic Quarterly, **9**: 144–146, 1942.
26. Hammer, H.: Primäre sarcomatose Ostitis mit chronischen Rückfallsfieber. Virchows Arch. f. path. Anat., **137**: 280–317, 1894.
27. Hauswirth, L., Rosenow, G., and Lansman, W.: Lymphosarcoma terminating in lymphatic leukemia (lymphosarcoma cell leukemia). Acta Haemat., **1**: 45–54, 1948.
28. Hargraves, M. M., Richmind, H., and Morton, R.: Presentation of two bone marrow elements; the "Tart" cell and the "L.E." cell. Proc. Staff Meet., Mayo Clinic, **23**: 25–28, 1948.
29. Henning, N., and Keilhack, H.: Die Ergebnisse der Sternalpunktion. Ergebn. d. inn. Med. u. Kinderh., **56**: 372–460, 1939.
30. Introzzi, P.: La biopsia de midollo osseo in Ferrata, A.; le Emopatie. Editrice Libraria Milano, **2**: 1376–1448, 1935.

31. Isaacs, R.: The bone marrow in anemia. Am. J. M. Sc., **193**: 181–191, 1937.
32. Jackson, H., Jr., and Parker, F., Jr.: Hodgkin's Disease and Allied Disorders. New York: Oxford University Press, 1947.
33. Jagic, N., and Klima, R.: Über die diagnostische Bedeutung der Knochenmarkpunktion. Wien. klin. Wchnschr., **1**: 363–367, 1937.
34. Jürgens, R., and Graupner, H.: Darstellung eines Entwicklungssystems der Thrombocyten, Zugleich ein Beitrag über das "Blutplättchenbild" der Blutkrankheiten. Folia Haemat., **57**: 263–282, 1937.
35. Kienle, F.: Die Sternalpunktion in der Diagnostik. Leipzig: Georg Thieme, 1943.
36. Klima, R.: Sternalpunktion und Knochenmarksbild bei Blutkrankheiten. Berlin: Urban and Schwarzenberg, 1938.
37. Klima, R., and Rosegger, H.: Zur Methodik der diagnostischen Sternalpunktion. Klin. Wchnschr., **14**: 541–542, 1935.
38. Krumbhaar, E. B.: Hodgkin's disease of bone marrow and spleen without apparent involvement of lymph nodes. Am. J. M. Sc., **182**: 764–769, 1931.
39. Landolt, R. F.: Eosinophiles Leukamoid und Lymphogranulomatose. Schweiz. med. Wchnschr., **25**: 1071—1075, 1944.
40. Leitner, S. J.: Die Intravitale Knochenmarksuntersuchung. Basel: Benno Schwabe, 1945.
41. Limarzi, L. R.: Clinical application of sternal puncture. Proc. Inst. Med. Chicago, **16**: 365–368, 1947.
42. Limarzi, L. R., and Bedinger, P. L.: A modified and improved sternal puncture needle. Am. J. Clin. Path., **18**: 913–915, 1948.
43. Limarzi, L. R.: Indications and limitations of sternal puncture. Bull. Chicago Med. Soc., **51**: 558–563, 1949.
44. Limarzi, L. R.: Diagnostic value of sternal marrow aspirations. Illinois M. J., **75**: 38–48, 1939.
45. Limarzi, L. R.: Evaluation of bone marrow concentration techniques; a modified method for the simultaneous preparation and staining of blood and bone marrow films. J. Lab. and Clin. Med., **32**: 731–740, 1947.
46. Limarzi, L. R., Paul, J. T., and Poncher, H. G.: Blood and bone marrow in infectious mononucleosis; a review of the literature and report of twenty-five cases. J. Lab. and Clin. Med., **31**: 1079–1100, 1946.
47. Limarzi, L. R., Jones, R. M., Paul, J. T., and Poncher, H. G.: Sternal marrow in Banti's syndrome and other splenomegalic states; the effect of splenectomy. Am. J. Clin. Path., **13**: 231–248, 1943.
48. Limarzi, L. R.: Thrombocytopenic purpura. M. Clin. North America, **28**: 153–186, 1944.
49. Limarzi, L. R., Poncher, H. G., and Birch, C. L.: The bone marrow in hemophilia during life; a study of the cellular elements and the coagulation time of the marrow blood. J. Lab. and Clin. Med., **31**: 777–784, 1946.
50. Limarzi, L. R.: Sternal puncture. American Practitioner, **2**: 514–522, 1948.
51. Major, R. H., and Leger, L. H.: Marked eosinophiles in Hodgkin's disease. J.A.M.A., **112**: 2601–2602, 1939.
52. Mallarme, J.: Le Myelogramme Normal et Pathologique par Ponction Sternale. Paris: Gaston Doin et Cie., 1938.
53. Markoff, N.: Die Beurteilung des Knochenmarks durch Sternalpunktion. Deutsche Arch. klin. Med., **179**: 113–133, 1936.
54. Medlar, E. M.: An interpretation of the nature of Hodgkin's disease. Am. J. Path., **7**: 499–514, 1931.
55. Mendell, T. H., Meranze, B. S., and Meranze, T.: The clinical value of sternal marrow puncture. Ann. Int. Med., **16**: 1180–1196, 1942.
56. Michels, N. A.: The plasma cell. Arch. Path., **11**: 775–793, 1931.
57. Middleton, W. S.: Some clinical caprices of Hodgkin's disease. Ann. Int. Med., **2**: 448–468, 1937.
58. Morrison, M., and Samwich, A. A.: Clinico-hematologic evaluation of bone marrow biopsies. Am. J. M. Sc., **198**: 758–773, 1939.
59. Nordenson, N. C.: Studies on bone marrow from sternal puncture. From the IV Medical Service, St. Eriks Hospital, Stockholm, Sweden, 1935.
60. Parkes-Weber, F., and Huber, H.: Hämatologische Beobachtungen. Deutsche Arch. klin. Med., **185**: 102–115, 1939.
61. Parof, J., Fischgold, H., Abaza, A., and Lewi, S.: Maladie de Hodgkin a localisation osseuse et cutanée (données de la ponction sternale). Sang, **13**: 797–803, 1939.
62. Paula e Silva, J. de: Contribuicao para o estudo da medula ossea na linfogranulomatose maligna. São Paulo méd., **2**: 83–110, 1943.
63. Piney, A., and Hamilton-Paterson, J. L.: Sternal Puncture: A Method of Clinical and Cytological Investigation. Ed. 2. New York: Grune and Stratton, 1944.
64. Propp, S., and Schwind, J. L.: Sternal puncture as practical diagnostic procedure. Ann. Int. Med., **21**: 580–606, 1944.

65. RASTELLI, M.: La Punctura Sternale. Metodica Diagnostica Emocitologica e Microbiologica. Roma: Iniezioni Endomidollari Edizioni Italiane, 1943.
66. REBUCK, J. W.: The structure of the giant cells in the blood-forming organs. J. Lab. and Clin. Med., **32**: 660–699, 1947.
67. REED, D.: On the pathologic changes in Hodgkin's disease with special reference to tuberculosis. Rep. Johns Hopkins Hosp., **10**: 133–196, 1902.
68. REVOL, L.: L'exploration de Moelle Osseuse par Ponction Sternal. Paris: J. B. Bailiere, 1938.
69. ROHR, K.: Knochenmarksmorphologie des menschlichen Sternalpunktates. Berlin: Urban and Schwarzenberg, 1937.
70. ROHR, K., AND HEGGLIN, R.: Tumorzellen im Sternalpunktat. Metastasennachweis maligner Gerschwültste in Knochenmark. Deutsche Arch. klin. Med., **179**: 61–79, 1936.
71. ROHR, K.: Knochenmarksmorphologie des menschlichen Sternalpunktates. Berlin: Urban and Schwarzenberg, 1937.
72. ROSENTHAL, N.: Blood Platelets and Megakaryocytes. In Downey, H.: Handbook of Hematology. Vol. 1. New York: Paul B. Hoeber, Inc., pp. 449–496, 1938.
73. ROTH, G. M., AND WATKINS, C. H.: The leukocyte picture in Hodgkin's disease. Proc. Staff Meet., Mayo Clinic, **11**: 593–597, 1936.
74. ROVERSI, A. S., AND TANTURRI, E.: La punctura dello sterno nella practica medica. Haematologica, **16**: 709–776, 1935.
75. SCHULTEN, H.: Die Sternalpunktion als diagnostische Methode. Leipzig: George Thieme, 1937.
76. SCOTT, R. B.: Sternal puncture in the diagnosis of diseases of the blood forming organs. Quart J. Med., **8**: 127–172, 1939.
77. SEELIGER, S.: Über Organbefunde und ihre Bedeutung für die Pathogenese bei essentieller Thrombopenie und Aleukie. Klin. Wchnschr., **3**: 731–735, 1924.
78. SPARLING, H. J., JR., ADAMS, R. D., AND PARKER, F., JR.: Involvement of the nervous system by malignant lymphoma. Medicine, **26**: 285–332, 1947.
79. STAHEL, R.: Die diagnostische Drüsenpunktion. Leipzig: Thieme, 1939.
80. STEINER, P. E.: Hodgkin's disease; the incidence, distribution, nature and possible significance of the lymphogranulomatous lesions in the bone marrow, a review with original data. Arch. Path., **36**: 627–637, 1943.
81. SUNDBERG, R. D.: Sternal aspirations. Staff Meet., Bull. Hospitals Univ. Minnesota, **17**: 389–404, 1946.
82. SUNDBERG, R. D.: Lymphocytogenesis in human lymph nodes. J. Lab. and Clin. Med., **32**: 777–792, 1947.
83. SYMMERS, D.: Mycosis fungoides as a clinical and pathologic nonexistent. Arch. Dermat. and Syph., **25**: 1–5, 1932.
84. SYMMERS, D.: Lymphoid diseases; Hodgkin's granuloma, giant follicular lymphadenopathy, lymphoid leukemia, lymphosarcoma and gastrointestinal pseudoleukemia. Arch. Path., **45**: 73–131, 1948.
85. TEMPKA, T., AND BRAUN, B.: Das morphologische Verhalten des Sternumpunktates in verschiedenen Stadien der perniciösen Anämie und seine Wandlungen unter dem Einfluss der Therapie. Folia Haemat., **48**: 355–401, 1932.
86. THADDEA, S.: Die Sternalpunktion und ihre klinische Verwertung. Stuttgart; Ferdinand Enke, 1943.
87. TISCHENDORF, W.: Klinische und morphologische Betrachtungen zur lymphogranulomatose. Deutsche Arch. klin. Med., **186**: 98–111. 1940.
88. TURNBULL: Quoted by Symmers.[84]
89. VARADI, S. M.: L'Infiltration lymphogranulomateuse du sternum; sur un cas de maladie de Hodgkin, diagnostique par ponction sternale. Sang, **12**: 106–110, 1938.
90. VELASCO MONTES, F.: Über die hämatologische Diagnose der Lymphogranulomatose (Hodgkinsche Krankheit). München. med. Wchnschr., **1**: 255–258, 1939.
91. VOGEL, P., ERF, L. A., AND ROSENTHAL, N.: Hematological observations on bone marrow obtained by sternal puncture. Am. J. Clin. Path., **7**: 436–447; 498–515, 1937.
92. VOGEL, P., AND BASSEN, F. A.: Sternal marrow of children in normal and pathologic states. Am. J. Dis. Child., **57**: 245–268, 1939.
93. WEERDT, W. DE: Recherches hematologiques sur la biopsie medullaire. Rev. belge sc. méd., **11**: 297–325; 337–414; 440–466, 1939.
94. WEIL, P. E., ISCH-WALL, P., AND PERLES, S.: Etude cytologique de la ponction sternale dans les hemopathies de l'adulte. Le Doumentaire Medical, **3**: 36–40, 1936.
95. WEIL, P. E., AND PERLES, S.: La Ponction Sternale. Paris: Masson et Cie, 1938.
96. WEINER, W., AND KAZNELSON, P.: Uber die zellige Zusammensetzung des Knochenmarkes nach Erfahrungen mittels der Sternalpunktion nach Seyfarth. Folia Haemat., **32**: 233–261, 1926.

97. WILLI, H.: Über den Bau und die Funktion der Megakaryocyten und ihre Beziehungen zur thrombopenischen Purpura. Folia Haemat., **53**: 426–439, 1935.
98. WINTROBE, M. M.: Clinical Hematology, Ed. 2. Philadelphia: Lea and Febiger, 1946.
99. WOLFF, J. R., AND LIMARZI, L. R.: Blood disorders associated with pregnancy; the value of sternal marrow biopsy. Am. J. Obst. & Gynec., **51**: 447–466, 1946.
100. WOLFF, J. R., AND LIMARZI, L. R.: Anemia in pregnancy; a correlation of the peripheral blood and bone marrow findings. J.A.M.A., **128**: 482–488, 1945.
101. WOLMAN, I. J.: Clinical applications of bone marrow examination in childhood. Am. J. M. Sc., **214**: 677–687, 1947.
102. YOUNG, R. H., AND OSGOOD, E. E.: Sternal marrow aspirated during life; cytology in health and disease. Arch. Int. Med., **55**: 186–203, 1935.
103. ZIEGLER, K.: Die Hodgkinsche Krankheit. Jena: Gustav Fisher, 1911.

SERUM LIPIDS FOLLOWING NEPHRECTOMY AND IN MERCURIC CHLORIDE POISONING*

DONALD H. ATLAS, M.D., MURRAY M. CASH, M.D., AND MARTIN M. KIRSCHEN, M.D.

From the Departments of Medicine and Pathology, The Chicago Medical School and Mount Sinai Hospital of Chicago

The mechanism of the disturbed lipid metabolism observed in the nephrotic stage of chronic glomerulonephritis and in the true lipoid nephrosis of children has been the subject of considerable speculation and investigation, but still remains obscure. It has been suggested that lipoid nephrosis is a primary disturbance of lipid metabolism,[1] and that the renal changes with subsequent hypoproteinemia and anasarca are secondary manifestations.

Others have taken an opposite view and believe that primary renal disease can, on occasion, result in hyperlipemia and hypercholesterolemia. The chief support for the latter view seems to stem from the reports of Heymann[4, 6] who produced hyperlipemia and hypercholesterolemia in dogs by unilateral and bilateral nephrectomy, and by subcutaneous injections of bichloride of mercury, uranium nitrate, and potassium bichromate. However, the oral administration of mercuric chloride to dogs and rats did not induce hyperlipemia, and carbon tetrachloride given orally to dogs periodically for 12 to 18 days prior to the subcutaneous injection of mercuric chloride inhibited the development of hyperlipemia.[5] These latter results indicate that liver injury can inhibit the development of hyperlipemia, otherwise reported as occurring regularly after parenteral administration of bichloride of mercury.

Farr, Smadel, and Holden[3] observed marked elevations in lipid fractions during the first two weeks of experimental nephritis produced in rats by injections of nephrotoxic sera. It should be noted that Heymann observed the effect of unilateral nephrectomy in only two dogs. He states that "in both animals, the level of blood lipids rose for from 4 to 7 days and then returned to normal in 12 or 14 days after the operation." He concludes from this that "the increase is obviously connected with the sudden removal of one kidney, while the return to normal level may well be due to the subsequent hypertrophy of the remaining kidney." Sham operations performed as controls produced no demonstrable effect on blood lipid levels.

He later found that the intravenous administration of Ringer's solution and glucose limited, but did not abolish, the extent of postnephrectomy hyperlipemia in dogs.[5] He concluded that "the tubular apparatus of the kidneys of dogs possesses a regulatory influence on the blood lipids such as has hitherto been unknown," and further states that "it is probable that the human kidney exerts the same function. . . ." In the same paper, however, Heymann reported no change in the total lipids, cholesterol and phospholipids (such as lecithin) following unilateral nephrectomy in three patients with hydronephrosis, pyohydro-

* Received for publication, July 15, 1949.

nephrosis and renal tuberculosis, respectively. He also made similar lipid studies on six patients who had ingested mercuric chloride. Four of these patients died in uremia. All lipid values in this series were normal or low.

Because of these conflicting results and the prevailing opinion in the literature and standard text-books that unilateral nephrectomy and bichloride poisoning produce hyperlipemia and hypercholesterolemia, additional studies of this type in human subjects are indicated.

MATERIAL AND METHODS

Total serum lipid, phospholipid, cholesterol and cholesterol ester determinations were made on five patients prior to, and at varying periods following, nephrectomy which was performed for a variety of reasons. Similar determina-

TABLE 1

VALUES OF SERUM LIPIDS BEFORE AND AFTER NEPHRECTOMY

AGE, COLOR, SEX OF PATIENT	PRE-OPERATIVE					FOLLOWING NEPHRECTOMY				
	Pathologic Diagnosis, Confirmed by Operation	Mg. per 100 ml. Serum				Mg. per 100 ml. Serum				Post-operative Day on Which Blood Was Drawn
		Chol-esterol	Chol-esterol Esters	Phos-pho-Lipids	Total Lipids	Chol-esterol	Chol-esterol Esters	Phos-pho-Lipids	Total Lipids	
65 W F	Carcinoma kidney with metastasis	274	188	18.4	860	295	174	14.5	960	1st
53 W M	Carcinoma ureter, hydronephrosis, pyelonephritis	219	175	16.0	800	169	106	10.5	1,010	3rd
49 W F	Pyelonephritis, suppurative	182	123	25.3	800	214	159	18.0	780	7th
53 W F	Pyelonephritis, chronic	280	182	17.5	840	235	140	15.0	680	3rd
14 W M	Ascending pyelonephritis, aberrant vessel, lower pole	207	129	19.0	800	160	111	17.0	140	1st

tions were also made on a patient who subsequently died in uremia from bichloride poisoning. The total lipids were determined by the method of Wilson,[8] the phospholipids by the method of Youngburg,[10] and the cholesterol and cholesterol esters by the method of Bloor.[2]

RESULTS

Unilateral nephrectomy performed on five patients produced no significant alterations of the serum total lipids, phospholipids, cholesterol or cholesterol esters. No alteration of lipid fractions were observed in the single case of fatal bichloride poisoning.

COMMENT

The increase of serum lipids following unilateral nephrectomy or bichloride poisoning reported in dogs was not demonstrable by us in human subjects. Our

results are in agreement with the more recent studies of Heymann and with the observations of Winkler and his co-workers[9] who reported no consistent increase in lipid components following unilateral nephrectomy in dogs. In two of their experiments, the values of all lipid substances increased two or three days after unilateral nephrectomy, while in a third, only cholesterol increased. They stated that in this group of dogs "all elements but cholesterol tend to return to normal within a week." In seven of their dogs, unilateral nephrectomy was without effect on the serum lipids during a three to ten-day postoperative period. These workers also reported no change in the serum lipids after unilateral nephrectomy in two Rhesus monkeys.

It may rightfully be questioned whether the results of the removal of a normal kidney should be compared with the results of removal of diseased kidneys. The closest approach to the experimental situation would be found in the removal of a traumatically ruptured but otherwise normal kidney in man. We have not as yet had occasion to study such cases.

TABLE 2

VALUES OF SERUM LIPIDS IN FATAL MERCURIC CHLORIDE POISONING

(Patient was a 37 year old woman who ingested 30 tablets of bichloride on March 2, 1949 and died of renal insufficiency on August 20)

DATE	CHOLESTEROL	CHOLESTEROL ESTERS	PHOSPHOLIPIDS	TOTAL LIPIDS
March 11	185	80	14.7	470
March 15	157	82	11.3	450

In all of our subjects varying degrees renal of function were found to be preserved, as demonstrated by concentration of dye on intravenous pyelography, visualization of the secretion of urine from the ureteral orifice of the affected kidney by cystoscopic examination, and on gross and microscopic study of the removed kidney. Even in the cases in which kidney function was reported to be almost normal, no effect on the lipids was observed following nephrectomy.

It is well known that the lipemia and hypercholesterolemia of the nephrotic stages of patients with chronic glomerulonephritis subside as renal insufficiency progresses, and in the most advanced stages of chronic glomerulonephritis, lower nephron nephrosis and nephrosclerosis, serum lipids are usually normal. Bell[1] states that "the increase of cholesterol seems to be caused by the depletion of plasma proteins" but does not elaborate on the mechanism. Loss of plasma proteins from other causes such as recurrent ascites does not produce hypercholesterolemia.

The theory that the increase in lipids in nephrosis is a compensatory attempt to maintain the osmotic pressure during the hypoproteinemia of nephrosis is untenable.[7] It is our opinion that there is unsufficient evidence to warrant the conclusion that primary renal disease can directly produce alterations in the blood lipids.

It seems probable that the commonly-stated hypothesis is correct, that in

nephrosis the rapid loss of proteins through the kidneys results in an increased mobilization of fats and proteins from depots to the liver for conversion to serum proteins, thus producing the so-called transportation hyperlipemia and hypercholesterolemia.

SUMMARY

1. No alteration in total lipids, phospholipids, cholesterol, and cholesterol esters was observed in the serum of five patients following nephrectomy.

2. No alteration in the value of these serum lipids was observed during the course of fatal mercuric chloride poisoning.

3. Increase in lipids following unilateral nephrectomy which has been reported to occur in dogs, was not found by us in human subjects.

Acknowledgments. We wish to thank Dr. I. Davidsohn for the use of the laboratories of the Department of Pathology and for assistance in the preparation of this report. We also wish to thank Dr. Leo A. Maslow of the Department of Urology for permission to study these patients.

REFERENCES

1. BELL, E. T.: Renal Diseases. Philadelphia: Lea & Febiger, p. 217, 1947.
2. BLOOR, W. R.: The determination of cholesterol in blood. J. Biol. Chem., **24:** 227–231, 1916.
3. FARR, L. E., SMADEL, J. E., AND HOLDEN, R. F., JR.: Observations on the occurrence of lipemia in rats with nephrotoxic nephritis, Proc. Soc. Exper. Biol. and Med., **51:** 178–179, 1942.
4. HEYMANN, W.: Renal hyperlipemia in dogs. Science, **96:** 163–164, 1942.
5. HEYMANN, W.: Further studies in renal hyperlipemia. Proc. Soc. Exper. Biol. and Med., **66:** 82–86, 1947.
6. HEYMANN, W., AND CLARK, E. C.: Pathogenesis of nephrotic hyperlipemia. Am. J. Dis. Child., **70:** 74–82, 1945.
7. POPJAK, G., AND MCCARTHY, E. F.: Osmotic pressures of experimental and human lipaemic sera; evaluation of albumin-globulin ratios with the aid of electrophoresis. Biochem. J., **40:** 789–803, 1946.
8. WILSON, W. R., AND HANNER, J. P.: Changes of total lipid and iodine number of blood fat in alimentary lipemia. J. Biol. Chem., **106:** 323–330, 1934.
9. WINKLER, A. W., DURLACHER, S. H., HOFF, H. E., AND MAN, E. B.: Changes in lipid content of serum and of liver following bilateral renal ablation or ureteral ligation. J. Exper. Med., **77:** 473–486, 1943.
10. YOUNGBURG, G. E., AND YOUNGBURG, M. V.: Phosphorus metabolism; a system of blood phosphorous analysis. J. Lab. and Clin. Med., **16:** 158–166, 1930.

LEIOMYOSARCOMA OF THE STOMACH

Report of Five Cases*

RAYMOND H. CONLEY, M.D., and JOHN H. OLWIN, M.D.

From the Department of Surgery, Veterans Administration Hospital, Hines, Illinois

A total of 98 cases of leiomyosarcoma of the stomach has been collected from the literature[11] up to 1948. The correct preoperative diagnosis of this condition is uncommon and it is the purpose of this paper to record one such case and, in addition, four other cases of clinically unrecognized gastric leiomyosarcoma collected from the files of Veterans Administration Hospital, Hines, Illinois.

The incidence of leiomyosarcoma in six different series of gastric sarcomas is indicated in Table 1. Of 16 cases of gastric sarcoma among patients at the Veterans Hospital, 5 (31 per cent) were leiomyosarcomas. The remaining 11 cases were classified as lymphosarcoma, 9 cases, fibrosarcoma, one case, and myosarcoma, one case.

The diagnosis of a leiomyosarcoma of the stomach may be difficult since the more malignant varieties may simulate tumors of epithelial origin.[8] Ewing[7] cautions against the interpretation as sarcoma any gastric tumor which grossly simulates carcinoma and which is associated with an ulcer. On the other hand, in the series of cases reported by D'Aunoy and Zoeller,[8] Brasch is quoted as stating that many cases assumed to be carcinoma or chronic gastric ulcer, associated with inflammatory change, on careful histologic re-examination will prove to be sarcoma. Pack and McNeer[10] and Ewing[7] classify leiomyosarcoma as outlined in Table 2. Leiomyosarcoma may be called spindle cell sarcoma, malignant leiomyoma, myosarcoma, myogenic sarcoma, or myoblastic sarcoma, and some cases may be confused with fibroblastic sarcoma, fusicellular sarcoma, fibrosarcoma, sarcoma of neurogenic origin, and malignant schwannoma (Cameron[4]).

Gastric leiomyosarcoma may be grossly classified as submucosal or endogastric, intramural, and subserosal or exogastric. The submucosal tumors usually are encapsulated, are not infiltrative, and involve the mucosa secondarily, producing an ulcer which may give rise to severe bleeding. Gastric leiomyosarcoma originates in the intramural portion of the stomach, as does gastric lymphosarcoma. The latter is characteristically flat, diffuse and infiltrating and may resemble linitis plastica. Leiomyosarcoma rarely remains confined to this layer. The subserosal leiomyosarcomas attain the largest size and frequently involve the surrounding viscera by compression rather than by invasion. These tumors often reach enormous size, may give the impression of inoperability, and form large necrotic masses with central degeneration and hemorrhage.

* Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration who assumes no responsibility for the opinions expressed or the conclusions drawn by the authors.

Received for publication, June 20, 1949.

Age and sex incidence. Leiomyosarcoma most commonly occurs between the fourth and fifth decades and, according to most authors, in equal proportion in males and females.[9, 13] Askey[1] and Pack and McNeer[10] found a predominance in males.

Pathologic features. Grossly the tumors are rubbery and expansible compared to carcinoma which is hard and infiltrative. Leiomyosarcomas rarely affect the orifices of the stomach to produce obstruction. They are usually sessile although pedunculation and cystic degeneration do occur. There is equal involvement of

TABLE 1

INCIDENCE OF LEIOMYOSARCOMA IN SEVERAL SERIES OF GASTRIC SARCOMA

AUTHOR	YEAR REPORTED	CASES OF GASTRIC SARCOMA REPORTED	INCIDENCE OF LEIOMYOSARCOMA
			Per Cent
D'Aunoy and Zoeller[6]	1930	135	5.9
Balfour and McCann[2]	1930	54	16.5
Pack and McNeer[10]	1935	9	44.0
Cameron and Breslich[4]	1941	104	37.0
Schroeder and Schattenberg[12]	1943	10	40.0
Conley and Olwin	1949	16	31.0

TABLE 2

CLASSIFICATION OF SARCOMA OF STOMACH

A. *According to Pack and McNeer*
 1. Spindle cell sarcoma
 a. Neurosarcoma
 b. Myosarcoma
 2. Lymphosarcoma
 a. Primary in stomach
 b. Generalized
 3. Miscellaneous round cell or alveolar sarcoma
 Metastatic sarcoma

B. *According to Ewing*
 1. Spindle cell myosarcoma
 2. Lymphomatous tumors
 3. Miscellaneous sarcoma of stomach

both curvatures, the lesion appearing most frequently in the region of the pylorus and to a lesser extent, in the cardia.[5] Microscopically, variations occur with the degree of differentiation. The cells may appear large and round, or spindle shaped, and are typically arranged in intertwining bundles. In the less malignant tumors the cells may be readily identified as of muscular origin, whereas in the more malignant growths they may easily be confused with round cell sarcomas or cells of epithelial origin. Metastasis is most frequent to the liver, with the formation of cystic tumors which may be multiple. Of the 39 cases reported by Cameron,[4] the incidence of metastasis was 20.5 per cent, only one

case metastasizing beyond the abdominal cavity. According to Chaffin,[5] in the unoperated patients in his series of 69 cases, 50 per cent died of causes attributable to the neoplasms, with metastasis present in only 12 per cent. Schindler *et al.*[11] reported metastasis in 15 per cent of their collected series. The common causes of death were hemorrhage, inanition, and suppuration of the mass.

Prognosis. The prognosis for gastric leiomyosarcoma is better than for gastric carcinoma.[12] According to Foot,[8] metastasis is slow and late, and cases of leiomyosarcoma run an average course of three years. Unlike carcinoma, the lesion does not involve neural sheaths and, therefore, produces little pain. In a series of 104 cases of gastric sarcoma reported by Cameron,[4] there were 6 five-year survivals, one survival among 41 spindle cell sarcomas, and 5 among 47 lymphosarcomas.

Symptomatology and clinical findings. The symptoms produced by this lesion vary with the location of the tumor. The most constant symptoms are pain simulating a peptic ulcer and responding to treatment for ulcer; hemorrhage resulting in hematemesis and melena; and a palpable abdominal mass. Nausea and vomiting, indigestion, and weight loss are not common until late in the disease. There is no correlation between gastric anacidity or hyperacidity and leiomyosarcoma. X-ray evidence of a filling defect showing a mucosal niche, demonstrated in the first case of this series, is typical. There seems to be some correlation between the frequency of gastric hemorrhage and the presence of a mucosal niche.

Treatment. The treatment for gastric leiomyosarcoma is surgical excision, since the lesion is radioresistant, in contrast to lymphosarcoma for which radiation is the treatment of choice.

REPORTS OF CASES

Case 1

A 62 year old woodworker was admitted to the hospital July 26, 1948. About six weeks previously, his fellow workers commented on his very pale appearance; at that time he felt well and was eating well. Three weeks later, while at work, he had a sudden attack of weakness, fainting and sweating. He took a cathartic following which he passed black stools. Subsequently his stools were almost constantly black. He complained of slight tenderness in the left lower quadrant of about three weeks' duration and an occasional feeling of abdominal discomfort which he attributed to "pressure against the heart from gas". There was no history of nausea, vomiting or food dyscrasia. The patient took no medication other than laxatives for constipation; he stated he had had constipation for years. His father died at 72 of "dropsy" and his mother, at 50 of carcinoma of the stomach. He had had no previous serious illnesses or operations, did not use tobacco, and drank only in moderation.

On examination he was well developed and well nourished, but pale. Blood pressure was 128/80 on admission. The abdomen was soft with slight tenderness in the left lower quadrant, the spleen and liver were not palpable, and there were no palpable abdominal masses.

FIG. 1. Case 1. Roentgenogram of stomach demonstrating rounded filling defect and central barium fleck; appearance characteristic of leiomyosarcoma.
FIG. 2. Case 1. Leiomyosarcoma of stomach with central ulcer.
FIG. 3. Case 1. Photomicrograph of leiomyosarcoma of the stomach. × 90.
FIG. 4. Case 1. Same lesion. × 550.

FIGS. 1–4

The patient's erythrocyte count was 1,470,000, the hemoglobin reading was less than 7 grams, the leukocyte count was 8600 and the total protein value was 5.5 grams. A gastric analysis at the time of gastroscopy revealed no free hydrochloric acid. Six transfusions, each of 500 cc. of whole blood, were given following admission and the patient was placed on an ulcer regimen with antacids. His stools became normal in color for two weeks, after which there was a two-day episode of black stools, followed again by passage of stools of normal appearance.

A gastrointestinal roentgen study August 3, 1948 revealed a rounded filling-defect (Fig. 1) measuring approximately 3.5 x 3.5 cm., lying in the antral region. Within the center of the defect near its superior lateral border, there was a large fleck of barium. The gastric contour on either side of the defect was normal in appearance and no pedicle was seen. Deformity and irregularity of the duodenal bulb were seen but no definite ulcer niche. A 50 per cent gastric residue was present after one hour, and 20 per cent after six hours. Diagnosis: Polypoid neoplasm of stomach with ulceration; suggestion of an ulcer of the duodenum; possible leiomyoma or leiomyosarcoma.

Gastroscopic examination August 17, 1948 by Dr. M. A. Spellberg revealed a smooth, spherical mass about 4 x 3 cm., projecting into the lumen of the stomach and coming into view in the antrum during inspiration. The dome of the mass revealed an oval ulceration about 1 cm. in diameter surrounded by a red areola. The base of the ulcer was covered by a dirty greenish brown exudate. The gastric mucosa was slightly hyperemic but otherwise normal. Impression: Benign leiomyoma of the gastric antrum.

Abdominal exploration was carried out on August 25, 1948. At this time a rounded, circumscribed mass about 2.5 cm. in diameter was found on the anterior surface of the greater curvature of the stomach about 3 cm. proximal to the pyloric ring. No local or distant metastases were evident. Sleeve resection of the stomach including the mass was carried out. The specimen consisted of a segment of stomach 10 x 5 cm. in its greatest diameters, with a circumscribed friable mass in the center, well demarcated from the mucosa and involving the muscularis. The mass (Fig. 2) measured 2.5 cm. in diameter and showed an area of superficial central ulceration about 1 cm. in diameter. Histologic examination (Figs. 3 and 4) revealed a cellular tumor having spindle to polyhedral-shaped cells which in some areas were arranged in interlacing bundles. The nuclei were hyperchromatic, of different sizes, and had occasional mitotic figures. Minute patchy areas of myxomatous change were seen. Microscopic diagnosis: Leiomyosarcoma.

The postoperative course was relatively uneventful except for serum hepatitis which developed September 20, 1948, and gradually subsided prior to the patient's discharge. Gastrointestinal roentgen studies postoperatively showed 25 per cent retention in the six-hour films, and the patient complained of slight epigastric distress immediately following a large meal. This subsided, however, and five months following surgery the patient was at work and asymptomatic.

Case 2

A 44 year old machinist was admitted to the hospital July 2, 1940. In December 1939 he first noted a slight cough which he did not consider to be serious. In March 1940 he developed pleurisy following a chest cold and lost 42 pounds in weight. His father died at 44 of pneumonia and his mother, at 63 of a stroke. On physical examination he appeared acutely ill and weighed 140 pounds. There was marked tenderness on the left side of the chest from the second to the fifth ribs, about two inches lateral to the sternum. Breath sounds and vocal and tactile fremitus were reduced over this area. The left axillary lymph nodes were enlarged and tender. The abdomen was essentially normal.

At the time of admission the blood count, urinalysis, and serologic test for syphilis were not abnormal. A smear of the sputum was negative for tubercle bacilli. Roentgen studies of the chest revealed a shadow of increased density involving the middle and outer zones of the left lung extending from the level of the third to the fifth ribs anteriorly. A heavy shadow of increased density was also noted at the left base, posterior to the heart. The

roentgenogram was characteristic of either encapsulated fluid or of a pleural tumor. A gastrointestinal roentgen series July 26, 1940 revealed a deformity of the pars media of the stomach which was diagnosed as carcinoma. Another roentgenogram August 2, 1940 revealed evidence of a defect 5 cm. in diameter on the posterior gastric wall near the lesser curvature approximately 10 cm. from the fundus. This resembled a penetrating ulcer. Because of the size and deformity, a diagnosis of new growth was made. Material aspirated from the left pleural cavity was diagnosed on biopsy as undifferentiated malignant tumor, probably carcinoma, primary site undetermined. The patient expired September 20, 1940.

Autopsy revealed a raised whitish yellow tumor 5 cm. in diameter on the posterior wall of the fundus of the stomach with slight central ulceration. A similar grayish white firm tumor infiltrated the thoracic wall of the left chest. Metastases were found in the lungs, pericardium, heart, mesenteric lymph nodes, gallbladder, liver, peritoneum, vertebrae and ribs. Microscopic examination revealed irregular bands of elongated cells resembling smooth muscle fibers. The nuclei were elongated with rounded ends and contained hyperchromatic closely-placed chromatin. Mitotic figures were numerous. Diagnosis: Leiomyosarcoma, grade 3, of stomach.

Case 3

A 51 year old Negro ice man was admitted to the hospital March 29, 1940, complaining of retrosternal pain of one week's duration, fullness in the chest, difficult swallowing of three weeks' duration, loss of twenty pounds in weight in the past month, and difficulty in sleeping on his left side. He gave a history of drinking one pint of whiskey daily and of having had a penile lesion in 1922. His father died of malaria, his mother of old age, and one brother died of syphilis. He knew of no other familial diseases.

On physical examination he was fairly well developed and poorly nourished. His pulse rate was 120 per minute and his blood pressure 106/70. There was mild engorgement of the neck veins with no pulsation evident. The cardiac examination was negative. There were moist rales in the bases of both lungs. The liver was enlarged two fingersbreadth below the costal margin and was tender on palpation. No ascites was evident. The abdomen was flat and soft and there was no rigidity.

Laboratory examinations disclosed an erythrocyte count of 4,290,000, a leukocyte count of 9000 and a hemoglobin value of 80 per cent. The Wassermann test was positive and the Kahn test, 3 plus. Roentgenograms of the chest showed no enlargement of the heart but prominence of the aorta. An electrocardiogram was interpreted as showing evidence of "myocardial disease".

An admitting diagnosis of syphilitic aortitis with aneurysm was made and the patient expired May 11, 1940 following a massive hemorrhage into the esophagus. A ruptured aortic aneurysm was found at autopsy. Five centimeters below the cardia on the greater curvature of the stomach there was an ulcerated tumor 12 x 8 cm., of "cauliflower appearance". It involved all layers of the stomach. There were metastatic nodules 1 to 8 cm. in diameter, present in the liver. Microscopic examination of the gastric lesion disclosed normal mucosa. The submucosa contained a mass made up of numerous irregular elongated cells with hyperchromatic nuclei rounded at the ends. The interlacing character of the cellular bundles suggested the structure of smooth muscle, and mitotic figures were fairly numerous. Diagnosis: Leiomyosarcoma of stomach.

Case 4

A 59 year old white male chef was admitted to the hospital November 7, 1935 complaining of general weakness and drawing pain in the right upper quadrant of the abdomen. Seventeen days prior to admission he stated that he suddenly began to perspire profusely, became nauseated and was seized with severe epigastric pain which radiated to the back. He was told he was very pale. The pain then seemed to localize in the right side of the abdomen. There was no history of previous illness and the family history was negative for

neoplasm. The patient admitted a loss of twenty pounds in weight in the last two months. He had no knowledge of the presence of a mass in the abdomen, blood in the stools or urine, or jaundice.

Physical examination revealed a well nourished man with a large mass in the abdomen which was considered to be the liver. It extended from the costal margin on the right to the rim of the pelvis, and 6 cm. below the left costal margin. There were some irregular nodules palpable on the surface of this mass. A reducible umbilical hernia was also present. Blood count, urinalysis, and serologic tests for syphilis were not abnormal. Roentgenograms of the heart and lungs were normal. Roentgenography of the colon revealed downward displacement of the hepatic flexure and of the transverse colon, apparently by the liver.

On November 21, 1935 an abdominal exploration was made and the umbilical hernia was repaired. A very large liver, slightly darker in color than normal, was noted. On its anterior and posterior surfaces were several firm nodules, the largest measuring 3 cm., in diameter. These nodules were covered by liver tissue but were distinctly elevated and firm, and afforded the impression of metastatic carcinoma. There was a small papillary mass about 3 mm. in diameter in the serosa of the anterior wall of the stomach. This did not have the appearance of a malignant neoplasm but it was removed and sent to the laboratory for examination. There was no other evidence of neoplasm in the abdomen. The patient expired on the third day.

Pathologic report: "Microsection of the tissue removed from the wall of the stomach revealed bundles of smooth muscle tissue cells arranged in strands and whorls. An occasional mitotic figure was seen. Diagnosis: Leiomyosarcoma of stomach wall with metastasis to liver."

Case 5

A 35 year old garage mechanic was admitted to the hospital January 10, 1932, complaining principally of epigastric pain which radiated to the back, vomiting, poor appetite, constipation, and weight loss. He stated that he first began to have stomach trouble eight to ten years prior to admission, his principal complaint being formation of gas and constipation. At that time he began taking cathartics daily. Epigastric pain appeared about three years prior to admission and was accompanied by nausea and vomiting. The vomiting would sometimes occur immediately after meals, particularly if he drank a glass of water following the meal. He also had vomiting whenever his work required him to be in a position that caused extra pressure to be exerted on the abdomen. He stated that he had never vomited any blood. In the past two years he had lost 43 pounds of weight.

Physical examination revealed an acutely ill, emaciated white man whose skin had a waxy appearance. The liver extended below the costal margin. The abdomen was tense and the slightest pressure induced pain, the tenderness being most acute in the epigastrium. A definite tumor mass could be palpated. The erythrocyte count was 4,750,000 and the leukocyte count, 8300. The hemoglobin was 70 per cent and the serologic test for syphilis was negative. The gastric content was described as having a foul odor, and contained a few pus cells, many large bacilli, no free hydrochloric acid, and total gastric acidity of 5 degrees. Gastrointestinal roentgen studies revealed the stomach to be fish-hook in shape with a constriction in the central portion, giving it an hour-glass deformity. There was a notch resembling an ulcer on the lesser curvature in the constricted area.

The patient was admitted in a moribund state and expired 13 days afterwards following a massive hemorrhage from the rectum.

Autopsy revealed about 300 cc. of fluid in the peritoneal cavity, a large mass in the left upper quadrant, but no generalized peritoneal involvement. About one-third of the left lobe of the liver was digested and was involved by massive necrosis. The adjacent anterior wall of the stomach was destroyed. The pancreas was surrounded by a tumor mass which extended from the stomach. The entire anterior wall of the stomach was completely necrotic and involved in a tumor mass that measured 30 x 12 cm., was made up of grayish white tissue, was quite firm and rather cartilaginous in consistency, and had a glassy ivory sheen to the

cut surface. The reaction was completely walled in by the diaphragm, the omentum, and the liver. The cavity thus formed was filled with blood and necrotic tumor tissue. There was no evidence of metastasis noted. The remainder of the intestinal tract was filled with partly digested blood, but the site of the hemorrhage could not be determined. However, it was thought to be somewhere in the stomach wall. Although the gross diagnosis was adenocarcinoma of the stomach with massive necrosis of the anterior wall and extension locally to the regional lymph nodes, the histologic diagnosis was sarcoma, spindle-cell type, probably of muscle origin.

SUMMARY

The literature pertaining to leiomyosarcoma of the stomach is briefly reviewed.

Five instances of this disease are reported. One case was correctly diagnosed pre-operatively.

REFERENCES

1. Askey, E. V., Hall, E. N., and Davis, K. S.: Sarcoma of the stomach. West. J. Surg., **39:** 839–847, 1931.
2. Balfour, D. C., and McCann, J. C.: Sarcoma of the stomach. Surg. Gynec. and Obst., **50:** 948–953, 1930.
3. Boyd, W.: Surgical Pathology. Ed. 6. Philadelphia: W. B. Saunders Company, p. 250, 1947.
4. Cameron, A. L., and Breslich, P. J.: Primary sarcoma of the stomach. Tr. West. S. A., **49:** 333–356, 1940
5. Chaffin, L.: Smooth muscle tumors of the stomach; review of literature with report of case (leiomyosarcoma). West. J. Surg., **46:** 513–524, 1938.
6. D'Aunoy, R., and Zoeller, A.: Sarcoma of the stomach; report of four cases and review of the literature. Am. J. Surg., **9:** 444–464, 1930.
7. Ewing, J.: Neoplastic Diseases; A Treatise on Tumors. Ed. 4. Philadelphia: W. B. Saunders Company, p. 285, 1940.
8. Foot, N. E.: Identification of Tumors. Philadelphia: J. B. Lippincott, p. 136, 1948.
9. Horsley, G. W., and Berger, R. A.: Leiomyosarcoma of the stomach; report of three cases. Ann. Surg., **112:** 22–30, 1940.
10. Pack, G. T., and McNeer, G.: Sarcoma of the stomach; report of nine cases. Ann. Surg., **101:** 1206–1224, 1935.
11. Schindler, R., Blonquist, O. A., Thompson, H. L., and Pettler, A. M.: Leiomyosarcoma of the stomach; its roentgenologic and gastroscopic diagnosis and its possible relation to pernicious anemia. Surg., Gynec. and Obst., **82:** 239–252, 1946.
12. Schroeder, G. F., and Schattenberg, H. J.: Sarcoma of the stomach; a clinical and pathologic study. Arch. Surg., **47:** 8–19, 1943.
13. Wolman, B.: Case of sarcoma of the stomach simulating splenic tumor. Brit. J. Surg., **34:** 322–323, 1947.

BONE FORMATION IN THE HEART*

ALBERT J. FINESTONE, M.D.,† AND CHARLES F. GESCHICKTER, M.D.

From the Department of Pathology, Georgetown University School of Medicine, Washington, D. C.

Primary massive calcification with ossification of the myocardium is a rare occurrence. Recently, we have had an opportunity to study such a case. In addition to the report of this unusual pathologic finding and a review of the literature, this communication offers a general consideration of heterotopic calcification and ossification.

Morgagni[28] as early as 1762 briefly mentioned a case of calcification of the heart. Topham[38] in 1906 cited a case of "bone formation in the heart", but recorded no microscopic examination. Oppenheimer[31] in 1912 reported the presence of "calcific and osteogenic change in the left auricle". He stated that the only previous case of bone in the heart was by Poscharissky[34] in 1905. In 1924, Scholz,[37] in the first comprehensive article on the subject of calcification of the heart reviewed the literature and considered many of the etiologic theories. He also emphasized the roentgenologic aspects and added a case of his own which was the first in which the premortem roentgen diagnosis was confirmed by autopsy. In the same year 2 cases of extensive deposits of calcium in the myocardium were cited by Cutler and Sosman.[8] Davidson[9] in 1928 mentioned a case of coronary infarction followed by small calcific granules at the site of the infarct. In 1932 Diamond[10] reported calcification of the myocardium in a premature infant; its occurrence in such a young subject was considered by the author to be significant in the pathogenesis of the calcification.

Bishop and Roesler[2] in 1934 combined 3 additional case reports with an excellent consideration of the roentgenologic diagnosis of intracardiac calcification. In the same year Moore[26] and Hirschboeck[15] each reported a case of myocardial calcification. In 1935 Niehaus[30] cited an unusual case of obstruction of a coronary artery due to pressure from a small calcified nodule in the myocardium. Yater and Cornell[40] collected the established cases of complete heart block reported in the literature and stated that in 9 of the 47 collected cases, including one of their own, the auriculo-ventricular dissociation was due to calcareous or fibrocalcareous lesions of the bundle of His. The case of Cohen and Levene[7] is the first in recent years in which bone formation has been reported in myocardial calcification. Parkinson, Bedford and Thompson[33] in 1938 assembled 16 cases of cardiac aneurysm and found 3 which showed calcification in the wall of the aneurysm. In 1940 Brown and Evans[4] in their case report called attention to the differences between primary massive calcification and other calcifications in the heart. Borman[3] and most recently Edelstein[11] have each reported a case of myocardial calcification with ossification.

* Received for publication, June 20, 1949.

† Present address, Temple University Hospital, Philadelphia, Pa.

REPORT OF CASE

Clinical Data

J. S., a 63 year old Negro man, was admitted to Georgetown University Hospital on October 14, 1948 with the chief complaints of fever and pain in the left leg. The patient was working and apparently well until 10 days prior to admission when he noted a vague pain in his left thigh, fever and generalized weakness. He had recently lost weight and had anorexia. The past medical history was not informative. Physical examination revealed marked dehydration and emaciation. The rectal temperature was 103.4, the pulse rate 100 per minute, respiratory rate 32 per minute and blood pressure 126/68. The apical impulse was diffuse in the fifth left intercostal space 9 cm. from the midsternal line. The heart sounds were distant, frequent extrasystoles were heard, but no murmurs. The chest was emphysematous and on auscultation of the lungs bilateral basal inspiratory crackling rales were heard. The left foot and calf were warm, slightly edematous and tender. The remainder of the physical examination was negative. The admitting diagnoses were left thrombophlebitis, arteriosclerosis, tuberculosis, malnutrition, and possible beriberi.

During the patient's hospitalization three urinalyses showed albumin and the highest specific gravity was 1.016. No significant anemia was present and the white count was 4200 with a differential of 27 per cent lymphocytes, 61 per cent mature polymorphonuclear leukocytes, 9 per cent young polymorphonuclear leukocytes and 3 per cent monocytes. The sedimentation rate on the day after admission was 10 mm. in one hour. The blood serology was negative and the nonprotein nitrogen was 56 mg. per 100 ml. blood. Agglutination tests with typhoid and paratyphoid organisms were negative as were two blood cultures. A venous pressure determination was normal. An electrocardiogram on the day after admission was interpreted as showing numerous auricular extrasystoles, infrequent ventricular extrasystoles, several combination beats, myocardial changes and intraventricular block. A chest x-ray film two days after admission showed diffuse mottling throughout both lung fields; there was no evidence of cavitation and the diaphragm was sharply outlined with no evidence of fluid; the heart was accentuated to the left and the aorta showed marked sclerosis. The conclusions were miliary congestion from heart failure with superimposed pneumonitis. Miliary tuberculosis could not be ruled out. On the fifth day of hospitalization, a definite bacteriologic diagnosis of tuberculosis was made. The patient demonstrated a septic type of fever the temperature varying between 101 and 104 degrees during his hospital course. Penicillin and Dicumarol were given for several days but were discontinued when a definite diagnosis of pulmonary tuberculosis was made and the left thrombophlebitis had subsided. Despite general supportive measures the patient became progressively weaker, and expired on the sixth day after admission.

Necropsy Findings

Gross description. Autopsy was performed five hours after death. Examination of the cranial cavity was restricted. Bilateral pleural adhesions were present and both lungs were studded with patchy areas of caseation. The pulmonary artery was opened *in situ* and showed no thrombus. The heart weighed 400 grams. The pericardium was not remarkable and the pericardial sac contained about 20 ml. of straw-colored fluid. The epicardial surface, right atrium and tricuspid valve appeared normal. In the lateral wall of the right ventricle a hard, calcific mass, 7 x 2 cm., was found extending from just below the tricuspid valve to within 2 cm. of the apex (Fig. 1). The medial aspect of this mass was incompletely covered by myocardium and endocardium. At the superior aspect of this otherwise roughly cylindrical mass was a projection, 1 x 0.5 cm., into the ventricular cavity just below, but not involving, the tricuspid valve. The wall of the right ventricle

measured 0.6 cm. in thickness but in the region of the mass it was 2.6 cm. thick. Cut section through this calcified area revealed normal-appearing myocardium surrounding the mass. On cross section the mass itself was roughly circular and had a definite white hard shell .4 cm. thick surrounding several small spaces which were filled with a reddish gelatinous material having the gross appearance of marrow. These spaces were separated from each other by dense trabeculae resembling the shell. The chordae tendineae and papillary muscles were not remarkable. The left ventricular wall measured 1.5 cm. in thickness. The mitral valve was slightly thickened and the aortic arch showed mild atherosclerotic changes. Dissection of the major branches of the coronary arteries revealed only mild atherosclerotic narrowing. The general configuration of this mass was revealed by a postmortem x-ray film of the heart (Fig. 2). The remainder of the autopsy examination was not remarkable.

Microscopic description. The gross impression of pulmonary tuberculosis was confirmed by microscopic examination. There was also microscopic evidence of tuberculosis in the spleen and liver. Examination of the myocardial mass showed a rim of relatively normal appearing cardiac muscle surrounding a definite layer of dense hyaline connective tissue which was undergoing calcification. The central area of the mass was composed of a meshwork of collagenous connective tissue and bone spicules (Fig. 3). Between this stroma were lakes of red cells and primitive myeloid cells. Other sections through the myocardium showed only occasional areas of interstitial fibrosis and plasma cell infiltration.

DISCUSSION

Pathologic calcification may be divided into two types, metastatic and dystrophic. In metastatic calcification calcium salts are deposited in tissues previously normal; it is usually associated with hypercalcemia. Hyperparathyroidism, either primary or secondary, destructive lesions of bone and hypervitaminosis D are etiologic factors in metastatic calcification.[29] These deposits occur chiefly in the kidneys, gastric mucosa and lungs. There is no evidence that the present case falls into this category. On the other hand, dystrophic calcification involves tissue changes, the term being applied to calcific deposits in dead or dying tissues. The mechanism by which the calcification occurs is still disputed. Several important observations have been made, however, and certain conclusions on this subject are justified.

In addition to inorganic phosphate, blood contains one or more phosphoric esters which have apparently not yet been completely identified. These organic esters are not ionizable and, therefore, do not increase the concentration of phosphoric ions. However, under the influence of the enzyme, phosphatase, hydrolysis of the ester occurs, with the release of phosphoric ions. These are then able to combine with calcium ions to form calcium phosphate. It was suggested by Robison[35] that the deposition of calcific material in the body might depend on some chemical reaction of this type. It is undoubtedly a more complex physicochemical mechanism since calcium is not separately precipitated in the form of calcium carbonate, calcium phosphate and other salts, but a complex calcium phosphate-calcium carbonate molecule is formed. Wells[39] found in examining



FIG. 1. Mass in wall of right ventricle, partially covered by endocardium and myocardium. Arrows indicate limits of mass.
FIG. 2. Postmortem x-ray film of heart showing general configuration of mass.
FIG. 3. Photomicrograph showing bone spicules. × 100.

these calcific deposits that the proportion of calcium phosphate and carbonate was quite constant and exactly the same as in normal bone.

Martland[25] found that when the pH fell hydrolysis of the phosphoric ester with the liberation of phosphoric ions occurred. With a shift to the alkaline side, synthesis of the phosphoric ester occurred, with a consequent reduction of free phosphoric ions. At the pH of blood (7.3 to 7.35) an equilibrium between synthesis and hydrolysis is established. Therefore, in order for calcification to occur, it is necessary to have phosphatase, an acid pH and a source of calcium. A local shift in pH can occur for many reasons. A mass of inert material which is not well vascularized can easily accumulate acid metabolites or, as stated by Jones and Roberts,[18] in any mesenchymatous tissue of low metabolism a decreased blood supply will lead to pathologic calcification. If phosphatase is present in the region of an acid pH, there will be hydrolysis of phosphoric esters with liberation of phosphoric ions which are then able to combine with calcium. Rous[36] has effectively summarized recent thought on this subject. The phosphatase activity of tissues which become the site of pathologic calcification has not yet been estimated except in the case of the kidney. However, in cases of heterotopic ossification of the abdominal wall, experimentally induced in dogs by transplanting bladder epithelium, Huggins[16] has shown that the ossifying tissue possesses a very high phosphatase activity.

As far as heterotopic bone formation is concerned, it can be stated that the osteoblast can no longer be regarded as a cell endowed with the specific power of laying down bone. Any of the connective tissues, after a preliminary de-differentiation to the state of primitive mesenchyme, may be built up again in the form of one of the other connective tissues. Leriche and Policard[23] and also Greig[14] state that the only factor necessary to determine bone formation in a mass of primitive mesenchyme is an excess of calcium and an adequate blood supply. This theory not only accounts for the development of skeletal bone but also heterotopic bone. Dystrophic calcific deposits may produce sufficient local irritation to cause increased vascularity. As stated above, this increased blood supply then converts the calcific mass into bone.

Heterotopic bone has been reported in almost every tissue of the human body[1, 5, 6, 12, 17, 20–22, 24, 27, 32] and also experimental heterotopic bone has been produced. Many and perhaps all of the cases of heterotopic calcification and ossification can be explained by this theory. For example, calcification sometimes occurs in the Achilles tendon after surgery at this site. It may be postulated that the reduced blood supply due to fibrosis produces the shift towards the acid pH which sets into operation the factors described above. The resultant calcific deposit may cause sufficient irritation to produce an increase in vascularity and bone formation may result. Also, the occasional discovery of bone in laparotomy scars may be explained on a similar basis. For these changes to occur, the various factors involved, phosphatase, phosphoric esters, calcium ions, pH and blood supply must be present in optimum quantities. These quantities have not yet been determined exactly and, therefore, it is impossible to predict when dystrophic calcification or ossification will occur in a given site.

Calcification and even ossification in the heart is not unusual. However, it is rather rare for these changes to occur in the myocardium. The pericardium and the valves are the most frequent sites in the heart for these changes. We are of the belief that a similar mechanism is involved in all of these cases, regardless of site. For example, let us consider calcific disease of the aortic valve. Karsner and Koletsky[19] have concluded that with only rare exceptions, calcific disease of the aortic valve is the result of rheumatic cardiac disease. Further, the rheumatic valve during healing shows the production of masses of fibrous tissue which subsequently undergo pronounced contraction and hyalinization.[13] Thus, if the other criteria in addition to the diminished blood supply are met, actual calcification may occur. A similar mechanism probably accounts for pericardial calcification.

Many of the reports of myocardial calcification and ossification have attempted an etiologic classification on the basis of vascular, inflammatory and toxic factors. However, we believe that a diminished blood supply for any reason, plus the other necessary requisites, will produce calcification. If an increased vascularity occurs later, ossification may result. It is only possible to speculate on the original cause of fibrosis in this case, but the commonest etiologic factor would be previous myocardial infarction. The finding of primitive marrow cells in this case can be explained on the basis of metaplasia, since there is a common precursor of all of these mesenchymal cells.

SUMMARY

1. A case of massive calcification of the myocardium with bone formation is reported and the literature on the subject is reviewed.

2. The etiology of dystrophic calcification with particular reference to this change in the heart is discussed. Also the relationship of later ossification in these areas to increased vascularity is considered.

3. It is our belief that the mechanism in all dystrophic calcification and ossification, regardless of site is similar. This unusual finding in the myocardium is reported in order to emphasize the similarity of the process involved in all calcific changes in the heart, including the more common sites in the pericardium and aortic valve.

REFERENCES

1. Asami, G.: Heteroplastic bone formation in the Fallopian tube. Am. J. M. Sc., **160:** 107–118, 1920.
2. Bishop, P. A., and Roesler, H.: The roentgenologic diagnosis of intracardiac calcification. Am. J. Roentgenol., **31:** 1–15, 1934.
3. Borman, M. C.: Calcification of left ventricular infarction recognized during life. Ann. Int. Med., **18:** 857–865, 1943.
4. Brown, C. E., and Evans, W. D.: Primary massive calcification of the myocardium. Am. Heart J., **19:** 106–113, 1940.
5. Brumbaugh, H. L.: Tendinitis ossificans traumatica. J. Bone and Joint Surg., **14:** 953–957, 1932.
6. Buerger, L., and Oppenheimer, A.: Bone formation in sclerotic arteries. J. Exper. Med., **10:** 354–367, 1908.
7. Cohen, J. N., and Levene, H. S.: Calcification of the myocardium with bone formation. Arch. Int. Med., **60:** 486–493, 1937.
8. Cutler, E. C., and Sosman, M. C.: Calcification of the heart and pericardium. Am. J. Roentgenol., **12:** 312–320, 1924.
9. Davidson, T. W.: Two cases of cardiac infarction. Brit. M. J., **1:** 211–213, 1928.

10. Diamond, M.: Calcification in the myocardium of a premature infant. Arch. Path., **14:** 137–145, 1932.
11. Edelstein, J. M.: Primary massive calcification with ossification of the myocardium. Am. Heart J., **31:** 496–500, 1946.
12. Ewing, J.: Neoplastic Diseases. Ed. 4. Philadelphia: W. B. Saunders Company, pp. 221, 222, 1940.
13. Forbus, W. D.: Reaction to Injury. Baltimore: The Williams and Wilkins Company, p. 239, 1943.
14. Greig, D. M.: Clinical Observations on the Surgical Pathology of Bone. Edinburgh: Oliver and Boyd, 1931.
15. Hirschboeck, F. J.: Calcification of the myocardium following coronary occlusion. Am. Heart J., **10:** 264–267, 1934.
16. Huggins, C. B.: The phosphatase activity of transplants of the epithelium of the urinary bladder to the abdominal wall producing heterotopic ossification. Biochem. J., **25:** 728–732, 1931.
17. Jones, H. W., and Roberts, R. E.: Calcification of the pericardium. Brit. J. Radiol., **32:** 167–170, 1927.
18. Jones, R. W., and Roberts, R. E.: Calcification, decalcification and ossification. Brit. J. Surg., **21:** 461–499, 1934.
19. Karsner, H. T., and Koletsky, S.: Calcific Disease of the Aortic Valve. Philadelphia: J. P. Lippincott Company. 111 pp., 1947.
20. Kaufmann, E.: Pathology for Students and Practitioners. Philadelphia: P. Blakiston's Son and Company, pp. 285, 421, 1929.
21. Keith, A.: Concerning the origin and nature of osteoblasts. Proc. Roy. Soc. Med. (Sect. Surg.) **21:** 1–8, 1927.
22. Kretschmer, H. L.: Myositis ossificans following suprapubic prostatectomy. J. Urol., **20:** 477–479, 1928.
23. Leriche, R., and Policard, A.: The Normal and Pathological Physiology of Bone. St. Louis: The C. V. Mosby Company, 1928.
24. Mallinson, F. B.: Ossification of both Achilles tendons with fracture of one. Brit. M. J., **2:** 836–837, 1932.
25. Martland, M.: The phosphoric-esterase of blood at various hydrogen ion concentrations. Biochem. J., **19:** 117–121, 1925.
26. Moore, J. J.: Myocardial calcification. Am. J. Roentgenol., **31:** 766–769, 1934.
27. Moore, R. A.: A Textbook of Pathology. Philadelphia: W. B. Saunders Company, p. 69, 1944.
28. Morgagni: De Sedibus and Causis Morborum, 1762; epis. 27, art. 16, etc., cited by Scholz.[37]
29. Mulligan, R. M.: Metastatic calcification. Arch. Path., **42:** 177–230, 1947.
30. Niehaus, F. W.: Obstruction of coronary artery due to pressure from calcified nodule in the myocardium. J. A. M. A., **104:** 2171–2173, 1935.
31. Oppenheimer, B. S.: Calcific and osteogenic change of the left auricle in a case of auricular fibrillation. Proc. New York Path. Soc. (New Series), **12:** 213–216, 1912.
32. Outerbridge, G. W.: Non-teratomatous bone formation in the human ovary. Am. J. M. Sc., **151:** 868–887, 1916.
33. Parkinson, J., Bedford, E. D., and Thompson, W. A. R.: Cardiac aneurysm. Quart. J. Med., N. S., **7:** 455–478, 1938.
34. Poscharissky: Zeigler's Beiträge, **38:** 135, 1905, cited by Oppenheimer.[31]
35. Robison, R.: The possible significance of hexosephosphoric esters in ossification. Biochem. J., **17:** 286–293, 1923.
36. Rous, M. C.: Calcification, ossification and decalcification. Clin. Proc. **1:** 257–262, 1942.
37. Scholz, T.: Calcification of the heart; its roentgenologic demonstration. Arch. Int. Med., **34:** 32–59, 1924.
38. Topham, J. A.: Bone formation in the heart. Brit. M. J., **2:** 953, 1906.
39. Wells, H. G.: Chemical Pathology. Ed. 5. Philadelphia: W. B. Saunders, p. 494, 1925.
40. Yater, W. M., and Cornell, V. H.: Heart block to calcareous lesions of the bundle of His. Ann. Int. Med., **8:** 777–789, 1935.

RETICULO-EPITHELIOID CELL GRANULOMAS IN BONE MARROW IN HERPES ZOSTER

Report of Case*

EMIL MARO SCHLEICHER†

From the Department of Internal Medicine, Minneapolis General Hospital, Minneapolis, Minnesota

Herpes zoster is believed to be caused by a virus and little is known about the ability of this neurotropic agent to attack other systems or organs.

It is the purpose of this communication to demonstrate granulomatous lesions in the aspirated sternal bone marrow of a patient with herpes zoster.

REPORT OF CASE

A 56 year old woman suffered general malaise, pain, and fever ranging from 100 to 104 F., for three days. Roentgenograms of the chest were negative and routine agglutination tests and blood cultures were negative. The Mantoux test in a dilution 1:1000 was positive. The hemoglobin level was 16 grams; erythrocyte count 5.2 millions; leukocyte count 4300; neutrophils 57 per cent, lymphocytes 35 per cent, eosinophils 4 per cent, monocytes 4 per cent, thrombocyte count 58,000.

Because of the thrombocytopenia and appearance of petechiae on the lower extremities, sternal bone marrow was aspirated at the level of the second intercostal space. From this specimen which was taken four days after the onset of symptoms, 65 marrow particles were isolated.[4] These particles were prepared for histologic study and were serially sectioned.[3] Twenty-three of them showed granulomatous structures of the type shown in Figure 1. A second sample was obtained at the level of the third intercostal space 10 days after the appearance of typical subcutaneous vesicular lesions (two days after the first bone marrow aspiration). Many of the vesicular lesions were already in the process of healing but a few petechiae were still present. The thrombocyte count was normal. From this marrow sample which was obtained on the sixteenth day of illness, 86 particles were isolated. Four particles showed granulomas of the type shown in Figure 2. It was noteworthy that more than one granuloma (maximum 4 lesions) were seen in the infected particles of the first marrow sample, whereas only one granuloma was present in the infected particles of the second specimen.

The reticulo-epithelioid cell granulomas differed from the epithelioid granulomas of miliary tuberculosis,[3] Boeck's sarcoid,[1] brucellosis[1, 6] and infectious mononucleosis.[5] The central part of the herpes zoster granulomas (without giant cells) was formed by more or less hypertrophic reticulum cells and transitional stages between them and the epithelioid cells. Scattered throughout were single and small groups of plasma cells in various stages of maturation. The plasma cells could be traced to the reticulum by the presence of reticular plasma cells and small syncytia of reticulum-proliferating plasma cells. The reticular derivation of the plasma cells was substantiated in meticulously prepared imprint preparations which were stained with Wright's blood stain.[2] A few eosinophils,

* Received for publication, April 2, 1949.

† Parke, Davis and Company Research Fellow in Hematology.

neutrophils and small lymphocytes were present in the central part of the greater number of the granulomas. A more or less loose cuff of eosinophils surrounded the aforementioned structure. The rim diffused in the hyperplastic myeloid tissue. Thus the boundaries of the reticulo-epithelioid cell granulomas were in no instance sharply defined. No inclusion bodies or necrosis were seen.

The granulomas seen in the second marrow sample showed from one to three giant cells of different size. The latter had from one to many light-stained nuclei which were either round, elliptical, horseshoe shaped or almost rectangular. The nucleoli were blue and sharply defined. In a few giant cells the nuclei were arranged in a semicircle enclosing a pink-staining granular cytoplasm and a few various-sized clear vaculoles. Some epithelioid cells, eosinophils, neutrophilic polymorphonuclears and small mature plasma cells were scattered between the giant cells. A more or less narrow rim of small lymphocytes gave the structures a somewhat nodular appearance. In the periphery of this cuff of lymphocytes were a few eosinophils and small plasma cells. No inclusion bodies or necrosis were seen.

In both marrow samples there was no evidence of a decrease of the megakaryocytogenesis although it was believed that the proliferation of thrombocytes in the first specimen was below normal. Imprint preparations, stained with Wright's blood stain, served as the means for the evaluation of the thrombocytogenesis.

DISCUSSION

The transitory granulomatosis in the sternal marrow may be causally related to the disease, herpes zoster, for reasons discussed below. The first bone marrow sample was taken because of the thrombocytopenia and appearance of petechiae. The large number of granulomas seen in the first marrow sample suggests that the virus was diffusely disseminated whereas the few granulomas present in the second and larger specimen are interpreted as due to an actual decrease of virus activity rather than the aspiration of sparsely infected marrow tissue. This assumption is based on data gathered from multiple marrow samples obtained from patients who had diffuse disseminated disease of the bone marrow such as miliary tuberculosis, sarcoidosis and brucellosis. It could be shown that each sample yielded a comparable number of lesions, that is, when the quantity of marrow tissue obtained is the same and the loss of particles during the processing for histologic study is held at the minimum. It is obvious that the number of serial sections made is an important factor in the compilation of quantitative data.

A comparative study of bone marrow granulomas, such as those of tuberculosis, sarcoidosis, brucellosis and infectious mononucleosis, revealed that the histopathology of the granulomas in our patient with herpes zoster may be sufficiently distinctive to separate them from the aforementioned structures. The conspicuous proliferation of plasma cells is unique particularly during the early development of the granuloma. It is admitted, however, that differential diagnostic difficulties are posed when the proliferation of plasma cells is minimal and giant cells dominate the pattern. Because of refusal of the patient to permit

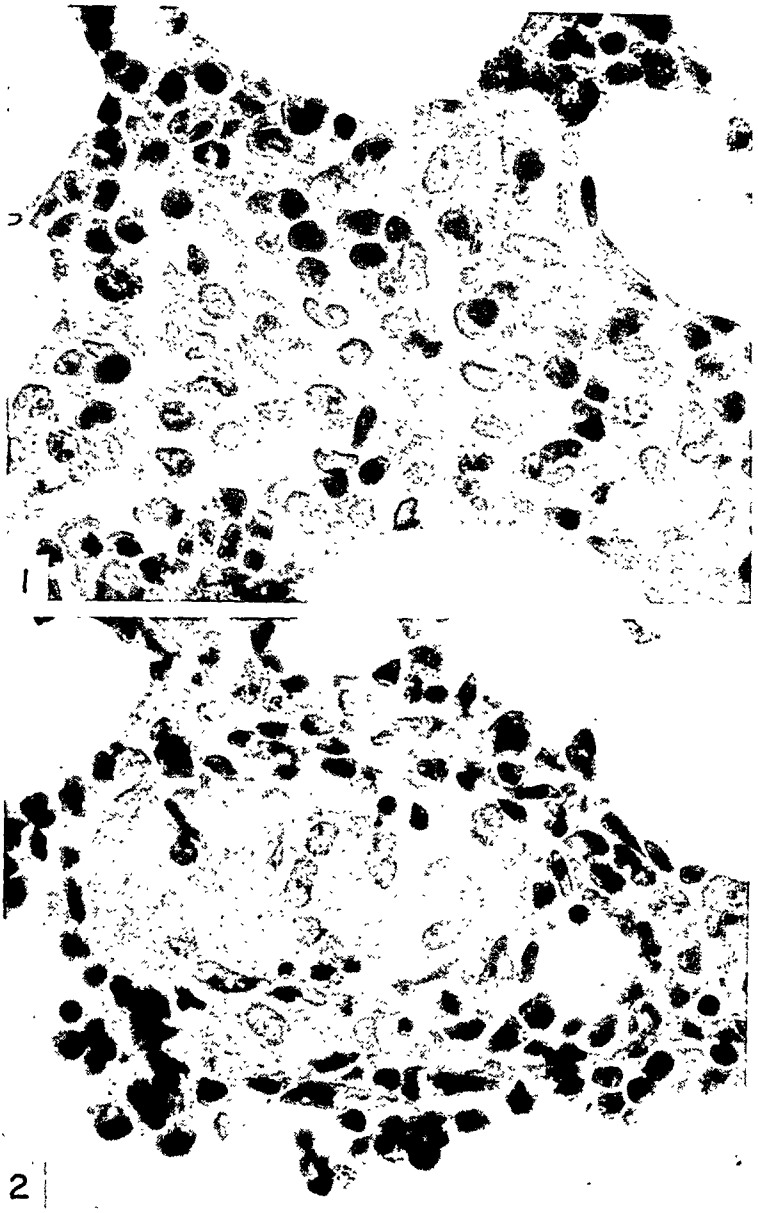


FIG. 1. Section from first sternal bone marrow specimen showing reticulo-epithelioid cell granuloma. Note development of plasma cells *in situ*. × 850.

FIG. 2. Section from second bone marrow specimen showing dominance of giant cells in granuloma. The loose peripheral rim is made up of small lymphocytes, mature plasma cells, eosinophils and myeloid cells in various stages of maturation. × 850.

more bone marrow aspirations, the time of the complete disappearance of the granulomas in the bone marrow could not be ascertained.

While the imprint preparations of several large marrow particles suggested

the presence of some kind of granuloma in the first marrow sample, no epithelioid or giant cells were observed in the imprints of marrow particles from the second sample. The discrepancy may be explained on the difference in the number of granulomatous lesions in the bone marrow at the time of sampling. Thus, histologic preparations of as many marrow particles as can be made available is essential, regardless of whether the pathologic state of the bone marrow can be ascertained from an examination of imprints or smears. A series of sections were stained for acid-fast bacilli because the patient had a positive Mantoux test but no organisms could be demonstrated.

No granulomatous lesions were seen in serially sectioned marrow tissue obtained from 5 persons with herpes simplex.

SUMMARY

1. A case is reported of herpes zoster with transitory thrombocytopenia, purpuric manifestations and reticulo-epithelioid cell granulomas in the sternal bone marrow.

2. In the sections of the first specimen of marrow, the granulomas were characterized by a conspicuous proliferation of plasma cells by the reticulum, while in the sections of the second specimen of marrow, the granulomas revealed the presence of various-sized giant cells. No inclusion bodies were seen.

3. The granulomatous lesions subsided with the healing of the cutaneous herpetic eruptions.

4. No granulomatous lesions could be demonstrated in the bone marrow of 5 patients with herpes simplex.

Acknowledgment. Thanks are expressed to Dr. G. Fahr, Head of the Department of Internal Medicine, Minneapolis General Hospital, for permission to publish this report.

REFERENCES

1. Gormsen, H.: The occurrence of epithelioid cell granulomas in human bone marrow. Acta med. Scandinav., Suppl. **213**: 154–164, 1948.
2. Schleicher, E. M.: Method for making imprints and direct smears from gross marrow units. Am. J. Clin. Path., **15** (Tech. Sect.): 8–9, 1945.
3. Schleicher, E. M.: Miliary tuberculosis of the bone marrow. Am. Rev. Tuberc., **53**: 115-121, 1946.
4. Schleicher, E. M.: Isolation of particles from aspirated sternal bone marrow for biopsy. Am. J. Clin. Path., **17**: 909-912, 1947.
5. Schleicher, E. M.: In press.
6. Sundberg, R. D., and Spink, W.: The histopathology of lesions in the bone marrow of patients having active brucellosis. Blood., Suppl. 1: 7-32, 1947.

ASYMPTOMATIC *SCHISTOSOMA JAPONICUM* INFECTION ASSOCIATED WITH RECTAL CARCINOMA

Report of a Case*

J. H. HILL, M.D.

From the Department of Pathology, University of Kansas School of Medicine, Kansas City, Kansas

This paper reports the occurrence of asymptomatic schistosomiasis in a veteran of the Pacific Theater in World War II who had a carcinoma of the rectum.

Articles dealing with coexisting cancer and *Schistosoma japonicum* infection are apparently to be found in the older Japanese literature, most of which was inaccessible. Kazama[12] in 1921 stated that intestinal cancer seemed more prevalent in regions infested with *S. japonicum*. In 9 cases of intestinal cancer he found old ova in the neoplasm, especially at the margin of the growth. He supposed that the mechanical and toxic injury from the ova may have led to irritation and malignant change. In 1926[13] he offered experimental evidence that *S. japonicum* infection played a role in the genesis of intestinal cancer. In 1930 Brumpt[3] reviewed the Japanese reports of rectal and colonic cancer associated with *S. japonicum* infection and concluded that the available statistics did not allow the conclusion that the irritant action of the ova produced cancer of the intestine. Ash and Spitz[1] stated that carcinoma of the large intestine not infrequently coexisted with *S. japonicum* infections, and showed malignant change in a polyp in a subject who had dead ova in a "cyst". Further details were not given.

Articles dealing with the pathology of infections with *S. hematobium* and *S. mansoni* which came from such diverse regions as Egypt,[7, 8, 10, 11] Brazil,[17] South Central Africa[9] and Puerto Rico[14] all indicate that the rectum, in spite of its frequent involvement, is rarely complicated by carcinoma. Schistosomiasis, except in Egypt, has not been reported to be associated with significant rectal papillomatosis. The explanation for this, according to Koppisch,[15] lies in the more severe infection resulting from the peculiar habits of the Egyptian fellaheen. However, this severe rectal papillomatosis apparently has no tendency to undergo malignant change.[6, 7]

Case reports of coexisting rectal cancer and schistosomiasis are few. In 1898 Kartulis[11] studied the pathologic anatomy of schistosomiasis in Egypt and found only a single case with primary rectal cancer. Roman and Burke[19] reported coexisting carcinoma of the colon and *S. mansoni* infection in an 18 year old Puerto Rican girl. Hutchison,[10] in his study of schistosomiasis in Egypt, found an instance of carcinoma of the rectum and another with carcinoma of the appendix. Diamantis[5] mentioned melanosarcoma of the rectum associated with *S. hematobium* infection. Koppisch[14] referred to a single case of cancer among 147 *S. mansoni* infections which were detected in 1009 consecutive necropsies

* Received for publication, July 5, 1949.

performed on native Puerto Ricans. This example occurred in a 16 year old boy who had numerous ova in the colon, retroperitoneal tissues, and in an obstructing colloid adenocarcinoma of the cecum.

REPORT OF CASE

Clinical notes. A 30 year old white man was admitted to the hospital on September 13, 1948, complaining of lower abdominal pain and constipation. In recent weeks the stools had diminished in caliber. Physical examination and a proctoscopic examination had revealed nothing remarkable, but radiologic examination revealed a stenosing lesion of the rectum. Routine blood and urine examinations were not unusual. A combined abdominoperineal resection of the rectum was performed following which the patient returned to his job as a mechanic.

Pathologic findings. Grossly the specimen consisted of the rectum and anus and measured 34 cm. in length. In the middle third an ulcerated growth caused stenosis of the lumen and appeared to be invading the perirectal fat. Just above the tumor was a polyp measuring 10 x 3 x 2 mm. Two poorly-defined areas of induration of the rectal wall were found about 5 mm. above the mucocutaneous junction and measured about 8 x 4 mm. Gross section through these areas revealed fibrous thickening of the submucosa. In the perirectal fat were three small lymph nodes, not remarkable grossly, together with a small fibrotic nodule 4 mm. in diameter.

Microscopically, the tumor was composed of atypical glands widely infiltrating the rectal wall and extending into the perirectal fat. Similar tumor tissue was found in the fibrotic nodule in the perirectal fat. The polyp was histologically benign. Sections of the two indurated areas in the rectal wall disclosed extensive fibrosis of the submucosa associated with numerous pseudotubercles (Fig. 1). These pseudotubercles had a sheath of epithelioid cells and lymphocytes and often contained one or more multinucleated giant cells of the foreign body type. The larger lesions had a fibrous capsule with central necrosis and a few polymorphonuclear leukocytes. Within and adjacent to the pseudotubercles were numerous ova many of which appeared viable while others were in various stages of disintegration. In some instances the ova lay free in the tissue spaces without exciting any apparent tissue reaction. Dr. E. C. Faust kindly studied the sections and was able to identify some as the ova of *S. japonicum*. Pseudotubercles were found in sections of the lymph nodes in the perirectal fat and a small lymph node lying next to the main tumor contained both metastasis of adenocarcinoma and the pseudotubercles (Fig. 2). Ova were not found in sections of the main tumor or polyp.

Diagnosis. Adenocarcinoma of the rectum; rectal schistosomiasis (japonicum); metastatic carcinoma and schistosomal granulomas in regional lymph node; benign rectal polyp.

FIG. 1. (upper). Photomicrograph of indurated area in rectum. Note the numerous ova of *S. japonicum* and the pseudotubercles in the submucosa. Hematoxylin and eosin. × 65.

FIG. 2 (lower). Photomicrograph of lymph node in region of main tumor. Note metastasis of adenocarcinoma and pseudotubercles containing degenerated ova. Hematoxylin and eosin. × 65.



FIG. 1



FIG. 2

Supplementary clinical data. After discovery of the schistosomal lesions it was found that the patient had served during World War II in the Pacific Theater with the U. S. Army. In December 1942, because of intermittent rectal bleeding which had begun before leaving the United States, he was operated upon for hemorrhoids while in New Guinea. While there, he had an attack of dysentery lasting one week and in October 1944 he experienced an attack of ureteral colic due to a calculus. In November 1944 he landed on the island of Leyte, P.I., remaining there until the middle of January 1945. While on Leyte he swam in the mouth of a fresh-water stream. One week later, the stream was placarded as containing snails capable of transmitting schistosomiasis. He did not notice unusual symptoms at this time and considered himself in fairly good health, even though he had continued to have intermittent rectal bleeding in spite of the operation for hemorrhoids. In August 1945 he was sent to a hospital because of infected tonsils. While there a proctoscopic examination was performed because of continued intermittent bleeding, but apparently nothing unusual was found.

Following his separation from the service in September 1945 the small rectal bleedings occurred at one to three month intervals. Subsequently, these bleeding episodes increased to twice a month. He noticed weakness after each episode and once was so weak he had to go to bed. Prior to his last hospitalization, however, he had had no recent rectal bleeding.

DISCUSSION

One of the two features of this case which attract attention is the lack of symptoms of the schistosomal infection. However, in 1916 Martinez[18] pointed out the absence of symptoms in 44 per cent of group of 220 patients with *S. mansoni* infection in Puerto Rico. In discussing the epidemic of schistosomiasis among American soldiers on the island of Leyte, Winkenwerder[20] mentioned the possibility of asymptomatic cases. This possibility was supported by the finding of Leavitt and Beck[16] of 34 cases of *S. japonicum* infection in a group of 177 apparently healthy military personnel who were on Leyte in the latter part of 1944. During World War II, Bercovitz *et al.*[2] found rectal ulceration in 60.7 per cent of 155 otherwise healthy Puerto Rican young men in whom routine fecal examination had revealed ova of *S. mansoni.*

The second interesting feature of this case is the possible connection between the rectal cancer and the rectal parasitism. To establish a connection requires either an accurate statistical study (which apparently has yet to be made) or suitable animal experimentation. The only animal experiment of this type seems to be that of Kazama[13] who produced an infection with *S. japonicum* in guinea pigs and after a time reported development of carcinoma with numerous ova contained in the tissue. Details of the work are not available and confirmation has not been reported. One instance can be mentioned however, in which parasites do apparently cause cancer, *i.e.*, the sarcoma of rats resulting from their infection with *Cysticercus fasciolaris,* the larva of the common tapeworm of the cat.[4]

It is not possible to be certain just when this patient began to experience his first symptoms of cancer. If the unexplained rectal bleedings were from the cancer, then it was present in 1942 and produced no serious complications for six years in spite of no treatment. This does not seem likely though it remains a possibility. It seems reasonable to believe he contracted schistosomiasis on Leyte, since he was adequatedly exposed and in residence during an epidemic. If his cancer resulted from the irritation of the schistosome ova in the rectum, then it developed in less than three and two-thirds years. No family history of cancer or polyposis of the colon could be found.

Cases with co-existing rectal cancer and schistosomiasis are too few to allow conclusions regarding age of the patient. In two other instances in which the age of the patient is stated, the ages were 16 and 18 years, a rather surprising finding.

CONCLUSIONS

An asymptomatic infection with *Schistosoma japonicum* was found in a veteran of the Pacific Theater in World War II who also had carcinoma of the rectum.

From the literature it appears that rectal carcinoma is a very infrequent complication of schistosomiasis, although additional cases must be studied to establish a causal relationship. Schistosomiasis, when asymptomatic, may be easily overlooked.

Acknowledgments. Dr. J. O. Ogilvie and Dr. C. C. Wilson kindly gave their permission to use the clinical data. Dr. E. C. Faust identified the ova in the sections.

REFERENCES

1. Ash, J. E., and Spitz, S.: Pathology of Tropical Diseases; An Atlas. Philadelphia: W. B. Saunders and Company, pp. 275 and 297, 1945.
2. Bercovitz, Z. T., Rodriquez, R., Hargrave, D. W., Dickie, J. D., and Green, C. E.: Studies on human Schistosoma mansoni infections; proctoscopic picture in asymptomatic schistosomiasis mansoni infections. J. A. M. A., **125:** 961–963, 1944.
3. Brumpt, E.: Rôle des bilharzies dans la production de certains cancers; étude critique à propos d'un cas nouveau. Ann. de parasitol., **8:** 75–101, 1930.
4. Bullock, F. D., and Curtis, M. R.: A study of the reactions of the tissues of the rat's liver to the larvae of Tenia crassicollis and the histogenesis of cysticercus sarcoma. J. Cancer Research, **8:** 446–481, 1924.
5. Diamantis, A.: Le cancer bilharzien vésical; à propos de onze cas personnels dont deux cas de cancer bilharzien vésical non infecté. J. d'urol., **40:** 408–432, 1935.
6. Dolbey, R. V., and Fahmy, I.: Bilharzial papillomatosis of the rectum. Lancet, **1:** 487–488, 1924.
7. Dolbey, R. V., and Mooro, A. W.: The incidence of cancer in Egypt. Lancet, **1:** 587–590, 1924.
8. Ferguson, A. R.: Associated bilharziosis and primary malignant disease of the urinary bladder, with observations on a series of forty cases. J. Path. and Bact., **16:** 76–94, 1911.
9. Gelfand, M., and Ross, W. F.: The diagnosis of schistosomiasis by rectal and vesical snips based on 150 autopsies. J. Trop. Med., **52:** 12–15, 1949.
10. Hutchison, H. S.: The pathology of bilharziasis. Am. J. Path., **4:** 1–16, 1928.
11. Kartulis, S.: Weitere Beitrage zur pathologischen Anatomie der Bilharzia (Distomum haematobium, Cobbold). Virchows Arch. f. path. Anat., **152:** 474–486, 1898.
12. Kazama, Y.: Über das Darmcarcinom bei Schistosomiasis japonica; zugleich eine genetische Beziehung zwischen seiner Entstehung und den Parasiteneiern. Gann; The Japanese Journal of Cancer Research, **15:** 22–24, 1921.
13. Kazama, Y.: A contribution to the clinical, histological and experimental knowledge on the causative connection between the development of carcinoma and the presence of the eggs of the Schistosomum japonicum (sixth communication on the studies of artificial carcinoma of the viscera). J. Hokuetsu Med. Soc., Vol. 41, No. 2, April, 1926. Abstr. in Japan M. World, **6:** 289, 1926.
14. Koppisch, E.: Studies on schistosomiasis mansoni in Puerto Rico; morbid anatomy of the disease as found in Puerto Ricans. Puerto Rico J. Pub. Health and Trop. Med., **16:** 395–455, 1941.
15. Koppisch, E.: Manson's schistosomiasis. J. A. M. A., **121:** 936–942, 1943.
16. Leavitt, S. S., and Beck, O. H.: Schistosomiasis japonica; report of its discovery in apparently healthy individuals. Am. J. Trop. Med., **27:** 347–356, 1947.
17. Magalhaes, A., and Coelho, B.: Cancer e esquistosomose. Bahia med., **12:** 7–16, 1941. Abstr. in J. A. M. A., **117:** 1049, 1941.
18. Martinez, I. G.: Investigations on the prevalence and clinical features of intestinal bilharziasis (schistosomiasis mansoni) in Puerto Rico. New Orleans M. and S. J., **69:** 352–394, 1916.
19. Roman, B., and Burke, A.: A case of carcinoma of the colon associated with schistosomiasis (bilharziosis) in a young woman. Am. J. Path., **2:** 539–544, 1926.
20. Winkenwerder, W. L.: Some observations of an epidemic of acute schistosomiasis which occurred in the Philippine Islands. Tr. Am. Clin. and Climatol. A., **58:** 153–170, 1946.



Etching by Kalman Kubinyi

DR. HOWARD T. KARSNER

EDITORIAL

HOWARD THOMAS KARSNER, M.D., LL.D.

Professor of Pathology, Western Reserve University, 1914–1949

Eleven years after graduation from medical school and four years after publication of his first scientific paper, Howard T. Karsner became professor of pathology at Western Reserve University and began the major phase of his fruitful career as a teacher, investigator and administrator. His productive scholarship is shown in the list of his publications: two textbooks, one of which has gone through seven editions; two monographs; four editions of a year-book of pathology; and one hundred and eight papers contributing to the scientific literature on many subjects. His interests are wide and varied: the pathogenesis of certain types of inflammation, studies of respiratory and circulatory disease, normal and abnormal humoral mechanisms, biochemical abnormalities in experimental disease, abnormalities in form and function of organs, morphology of tumors, problems of administration in medical school and laboratory, principles of teaching and the development of the experimental method in teaching, matters of importance in military medicine, essays on medical history and philosophy. No subject in human pathology has escaped his examination and direct, thoughtful comment. An enduring physical monument to his ambition and leadership is the Institute of Pathology of Western Reserve University, which he planned, found means to build and equip, staffed and directed. His students push on along the paths to which he led them: deans of medical schools, professors of pathology, teachers, experimentalists, and practitioners of their science. There have been few in this or any country with such a record. Now, after thirty-five years, unwilling to retire and count his honors, he has resigned to become research advisor to the Surgeon-General, United States Navy, a post to which he will bring all the wealth of high scholarship and mature judgment which he has accumulated in a long and industrious life.

FRANCIS BAYLESS

Cleveland

BOOK REVIEWS

Parathyroid Glands and Metabolic Bone Disease. Selected Studies. By FULLER ALBRIGHT, M.D., Assoc. Professor of Medicine, Harvard Medical School, Boston, Mass., and EDWARD C. REIFENSTEIN, JR., M.D., F.A.C.P., Consultant-in-charge, Department of Clinical Investigation, Sloan-Kettering Institute of Cancer Research, Memorial Hospital Cancer Center, New York. 393 pp., 157 figs., $8.00. Baltimore: The Williams & Wilkins Company, 1948.

In this compact volume, amounting to 311 pages of text (including 157 text figures), a surprising amount of information has been carefully and thoughtfully recorded.

Chapters I, II, III deal with the normal and pathologic physiology of the parathyroid glands, clinical hypoparathyroidism and clinical hyperparathyroidism, respectively. In these well-balanced sections the known and suspected actions of the parathyroid glands are authoritatively stated and interpreted with reference to clinical disturbances that are associated with either diminished or excessive production of parathormone. Chapter IV is assigned to the action of Vitamin D and dihydrotachysterol (A.T. 10).

In the remaining five chapters osteoporosis, osteomalacia, polyostotic fibrous dysplasia and Paget's disease are considered. The discussions of these disorders are well oriented with respect to the normal and pathologic anatomy and physiology of osseous tissues. Clinical signs and symptoms, and laboratory findings are presented in conjunction with roentgenologic and pathologic data that are illustrated frequently.

Because of their thorough familiarity with the subject matter, the authors have been able to bring together in this one volume most of the known anatomic, physiologic, and biochemical data that bear on the development and maintenance of osseous tissue. In addition they have been able to use these data skillfully in explaining the pathogenesis and progression of the disease processes to which the book is addressed.

Students of medicine should be encouraged to refer to this work in their basic science and clinical years. Physicians in general medicine or surgery should profit from familiarity with the contents of the book and any physician specializing in diseases of the skeletal systems should regard it as a must on the shelves of reference volumes.

Chicago G. A. BENNETT

Chemistry and Physiology of Growth. Edited by ARTHUR K. PARPART. 293 pp., illustrated. $4.50. Princeton, New Jersey: Princeton University Press, 1949.

The contents are a series of articles in the nature of reviews but also contain critical comments by their authors. They consist of:

I. Enzymes and the Synthesis of Proteins, by John H. Northrup
II. Molecular Morphology and Growth, by Francis O. Schmitt
III. Plant Growth Hormones, by Kenneth V. Thimann
IV. Unidentified Vitamins and Growth Factors, by Karl Folkers
V. The Kinetics of Growth of Micro-organisms, by C. B. van Niel
VI. Cellular Metabolism and Growth, by E. S. Guzman Barron
VII. Differential Growth, by Paul Weiss
VIII. Problems of Organization, by J. S. Nicholas
IX. Neoplastic Abnormal Growth, by C. P. Rhoads
X. The Adrenal Gland, a Regulatory Factor, by C. N. H. Long

Although the reviewer finds uneveness in the presentations, most are concise and to the point. A few contain, perhaps, more verbiage than might be needed; but if one wants a series of articles covering a number of fields in the growth problem, this book admirably serves the purpose. This can be said for both specialists in the various fields (for the reviewer has asked about this) as well as for the general reader.

To the pathologist this book should prove quite stimulating, for much of the knowledge treated can be directly applied to his everyday work. The theories expounded will help the

pathologist to orient his background of pathologic anatomy into the physiologic, physical, and to an extent into the chemical realms. Therefore, *Chemistry and Physiology of Growth* is recommended highly as an addition to the library of the pathologist for those hours in which he wishes to contemplate his specialty from numerous different approaches.

Philadelphia STANLEY P. REIMANN

Atlas of Peripheral Nerve Injuries. By WILLIAM R. LYONS, Ph.D., Associate Professor of Anatomy, University of California Medical School; and BARNES WOODHALL, M.D., Professor of Neurosurgery, Duke Medical School, Durham, North Carolina. 339 pp., 135 plates. $16.00. Philadelphia: W. B. Saunders Company, 1949.

This atlas is a valuable contribution to the understanding of surgery of peripheral nerve injuries and the neuropathologic processes underlying the repair or impossibility of repair in some lesions. The material was obtained at surgery by Woodhall and other Army neurosurgeons, and the anatomic and pathologic studies were carried out by Lyons. The material is unique and the observations extremely carefully made. The authors analyzed their cases from the points of view of primary and delayed sutures and compared the results. They correlated the results of the various methods used and the histopathologic aspects of the tissues removed in the course of each type of procedure. They also give an excellent discussion of the histologic and histopathologic features of the proximal and distal stumps of severed nerves as well as of the nerve both proximal and distal to the point of severance.

Grafts, both homografts and autografts, are said to have been a serious disappointment. There were only a very few successes, and these were achieved in short-length homografts. Various materials were used for these grafts; there was little to be preferred among the results secured in any one group.

There is a good balance between text and illustrations. The material is well organized. The type is clear and the paper excellent. The illustrations are well selected and reproduction is good.

Rochester, Minnesota JAMES W. KERNOHAN

Studies on Hookworm Disease in Szechwan Province in West China. By K. CHANG AND CO-WORKERS. 152 pp., 9 figs., 41 tables, 34 charts and graphs. $3.00. Baltimore: The Johns Hopkins Press, 1949.

This study on hookworm disease supported in part by the International Health Division of the Rockefeller Foundation was carried out in the Szechwan Province in Western China (Chungking is the capital) by Dr. K. Chang and co-workers as part of a program to determine the source of infection and the factors influencing the dissemination of hookworm, *Endamoeba histolytica*, and *Ascaris* and to suggest methods for control. Reports on the two latter named parasites are to be published in separate communications.

The studies were for the most part on farmers and their families engaged in the cultivation of different kinds of crops, chiefly mulberry trees (the leaves of which are used as feed for the silkworm), corn, sweet potatoes, rice, and a variety of vegetables. While the incidence of infestation with the hookworm was generally found to be quite high (99 per cent of the population in some localities), the degree or intensity of infestation varied considerably and was directly related to the extent of the use, and the intimate contact with, human feces used as fertilizer for these crops. The greatest number of clinically severe cases was found among the farmers engaged in the intercropping of corn and sweet potatoes, where after fertilizing the ground for the planting of corn with night soil, the sweet potato cuttings are planted by hand in the sandy soil following a rain. The warm, moist soil is ideal for the hatching of the innumerable ova. "Ground itch" on hands and feet is a common sight. The incidence and degree of infestation was much less among the rice growers.

In their conclusions, the authors emphasize the complexity of the problem of control. The ignorance, superstition, and poverty of the population in the agricultural areas can be overcome only after great effort and over a long period of time.

Educational propaganda in which the farmers are taught the cause of the disease from which they suffer, the value of the treatment which must be made available, and newer methods of agriculture designed to lessen the intimate contact with human excreta seems to be the most promising method of attack. Sterilization of the night soil is wholly impracticable.

The book should be of interest to any one having more than a passing interest in parasitology.

Rochester, New York W. S. THOMAS

Diagnostic Tests for Infants and Children. Principles, Clinical and Laboratory Procedures, Interpretation. By H. BEHRENDT, M.D. 529 pp., 45 figs. $7.50. New York: Interscience Publishers, Inc., 1949.

In applying diagnostic procedures to infants and children the physician is beset by a multitude of problems. The proper preparation of the subject for the test may be difficult. It may be impossible to obtain the cooperation of the patient. The collection of specimens may be a problem. The methods given in standard texts may call for larger samples than can be safely obtained. Special equipment may not be available. Interpretations are often hazardous because normal standards may be lacking or inaccessible or may be highly variable because of growth and other factors.

Dr. Behrendt's book attempts to meet these problems on a wide, perhaps too wide, a front. It contains information on a wealth of widely divergent subjects, such as gastric function-tests (including motility tests), hepatic and renal function-tests, carbohydrate metabolism, vitamin assays, circulating function-tests, skin tests for hypersensitiveness, electrocardiography, electroencephalography, hearing and sight, neuromuscular excitability, I.Q. and Rohrschach test, and endocrinologic methods, to name only a few. The enormous scope of the book suggests that large parts of it are based on reading and compilation rather than on actual experience in each field. One may question the necessity of combining so many unrelated topics in one volume. Few people interested in electroencephalography would consult the book for information on the Schick test and few readers concerned with the determination of vitamin C levels or urea clearance would find the section on psychologic tests useful to them. An even more compact and comprehensive book on laboratory procedures in pediatrics might have resulted if the author had confined himself to that subject in which he appears eminently qualified. This reviewer at any rate confesses to a lack of the truly encyclopedic knowledge required to deal with the work in its entirety and will confine his further remarks to the sections devoted to laboratory topics.

Dr. Behrendt has intelligently and authoritatively met a real need. His presentation is clear, concise and compact. Each chapter is not a mere outline of technical procedures but gives the physiologic background, methods of preparing the subject, collection of specimens, technical procedure, normal standards, a valuable section entitled "pediatric considerations" and a section on interpretation. The source material is critically chosen and the excellent bibliography at the end of each chapter is in itself a worthwhile contribution.

In dealing with procedures, technics suitable to the age group have been stressed with resulting emphasis on micromethods. However, macromethods are also described and a choice of methods is often given so that the book is of value to less highly specialized laboratories.

Lack of space prohibits a detailed discussion of each chapter. Inevitably the quality of different sections is uneven and minor omissions are frequent. The chapters dealing with chemistry and metabolism form the strongest part of the book. The section on Rh is already outdated. The omission of a chapter on hematology, while understandable from the author's avowed purpose of avoiding morphologic subjects, is regrettable because, in this field too, thinking has progressed along functional lines. Such functional hematologic tests as are included are dealt with sketchily. For example, the pitfalls in erythrocyte fragility tests resulting from lack of pH and temperature control are not even mentioned.

In spite of such relatively minor shortcomings, the book should prove of great value to every clinician, pediatrician, pathologist and medical technologist concerned with the diagnostics of disease in early life.

Detroit W. W. ZUELZER

Regional Ileitis. By BURRILL B. CROHN, M.D., Consulting Gastroenterologist, Mount Sinai Hospital, New York. 229 pp., 74 figs. $5.50. New York: Grune & Stratton, 1949.

This excellent new book carries on the classic work originally created when Crohn described the entity in 1932. Dr. Crohn has brought up to date the concepts as to theories of etiology and has excellently reviewed the historical background. He devotes several pages both to the gross and microscopic pathologic findings and includes excellent illustrations. The book goes into some detail as to the etiology, and the various theories are discussed objectively. The clinical features are well described and the course, complications and treatment, particularly of ileitis, are graphically handled. Considerable space is devoted to the roentgenographic study of regional ileitis and the technic of radiography. Differential diagnosis of the disease is also well treated. The treatment is considered under diet and medication as well as surgery. The author also tells of the combination of ileojejunitis and ileocolitis, the so-called combined forms, detailing the pathologic findings, etiology, and medical and surgical approach to treatment. The book has an ample group of references, and the author is to be greatly commended for bringing this complicated subject up to date in such a skillful and concise manner.

Oakland, California PAUL MICHAEL

Syphilis: Its Course and Management. By EVAN W. THOMAS, M.D., Professor of Clinical Medicine, New York University College of Medicine; Director, Rapid Treatment Center and Visiting Physician, Bellevue Hospital, New York: Consultant, United States Public Health Service. Foreword by John F. Mahoney, M.D., Director of Venereal Disease Research Laboratory, United States Public Health Service. 317 pp., 68 figs., $5.50. New York: The MacMillan Company, 1949.

This handbook stresses the immunology, course of untreated syphilis, interpretation of laboratory tests, and value of penicillin in treatment of the various stages of syphilis. The time-dose relationships of penicillin recommended are based on analysis of over 12,000 cases of syphilis treated with various schedules at the Rapid Treatment Center at Bellevue Hospital, New York City.

The chapters on the course of untreated syphilis and immunity of syphilis summarize information essential to the evaluation of the influence of treatment. The chapter on the interpretation of serologic tests for syphilis on blood and spinal fluid summarizes information essential to the diagnosis, treatment, and follow-up of syphilis. Generally speaking, if practicing physicians had a better appreciation of the information contained in this chapter, as well as in the chapter on principles underlying antisyphilitic treatment, their management of syphilis would be greatly improved.

The chapters on the diagnosis and treatment of early syphilis, latent syphilis, late benign syphilis, cardiovascular syphilis, neurosyphilis, syphilis in pregnancy, and congenital syphilis cover the essential features of diagnosis and end with a short summary of the penicillin treatment recommended. The chapter on neurosyphilis contains a clear, concise summary of the so-called Dattner-Thomas conception of the activity of neurosyphilis based on spinal fluid findings. This interpretation of spinal fluid findings, like his discussion of serologic fastness of the blood serum in Chapters 4 and 10, should do much to correct the present weakness of overtreatment in such cases. Kaplan's experiences, quoted in Chapter 10, with latent syphilis in New York State prisons is especially illuminating.

It is recognized that further experience with procaine penicillin may soon revolutionize the treatment of syphilis, and what is considered adequate treatment today may be obsolete tomorrow. This can readily be remedied by new editions of this inexpensive book. This volume provides the most authoritative and up-to-date summary available on modern

penicillin treatment of syphilis. Dr. Thomas might be considered an enthusiast for the penicillin treatment of syphilis. His recommended schedules of dosage are, however, conservative and the majority of syphilologists join with him in his enthusiasm. The book ends with an excellent chapter entitled The Public Health Aspects of Syphilis by Theodore J. Bauer, M.D., Chief of the Venereal Disease Division, United States Public Health Service.

This book fills an urgent need as an up-to-date summary in the rapidly changing status of modern management of syphilis. Its recommendations should be known to every physician who accepts the responsibility of treating syphilis.

Detroit LOREN W. SHAFFER

Rapid Microchemical Methods for Blood and CSF Examinations. By F. RAPPAPORT, PH.D., Laboratory Director of the Biochemical and Serological Department of the Beilinson Hospital, Petach Tiqva, and Laboratory Director of the Bacteriological and Serological Department of the Hadassah Municipality Hospital, Tel-Aviv, Israel. 404 pp., 72 figs., 32 tables. $8.75. New York: Grune and Stratton, Inc., 1949.

The book describes in adequate detail macro- and micro-methods for determining most of the normal and abnormal substances in blood and spinal fluid. After each procedure there is a short discussion of normal values and the interpretation of the results of the test. Also included are some tests more commonly considered to be hematologic or serologic, in nature, such as hematocrit and sedimentation rate determinations and cephalin-cholesterol flocculation and Takata-Ara reactions. The foreword and the preface state that the book evolved out of work done in Vienna and in Israel during the war. Because of the shortage of glassware during the war, many of the procedures were worked out with the use of pipettes or equipment made and calibrated by technicians. Though most chemists can do this, it is doubtful if many technicians in this country have the necessary experience.

Many of the methods are titrimetric, which should give accurate results in the hands of a qualified chemist; it is doubtful, however, if they would be accurate in the hands of a technician who is also expected to be proficient in most of the other fields of clinical pathology. Very few of the methods are adapted to the use of a photoelectric colorimeter, which is today almost standard equipment in laboratories in this country. Over sixty pages in the appendix are devoted to notes on the preparation of reagents, indicators and equipment, and have much of value for any clinical pathologist. The index is inadequate and does not include many of the tests described in the book. An excellent feature is the bibliography at the end of each chapter. An even more valuable feature, not found in most laboratory manuals, would be short notes on the accuracy of the methods suggested and a discussion of possible sources of error in the performance of the tests.

This book has value as a reference work, but it is doubtful whether many of the methods can be readily adapted to use in most clinical laboratories in this country.

Dearborn, Michigan H. J. LINN

Clinical Endocrinology for Practitioners and Students. By LAURENCE MARTIN, M.D., (Camb), F.R.C.P., (Lond), Physician to Addenbrooke's Hospital, Cambridge; and MARTIN HYNES, M.D., (Camb), M.R.C.P., (Lond), Reader in Medicine in the University of Cambridge. Foreword by Sir Lionel Whitby, C.V.O., M.C., M.D., F.R.C.P., D.P.H., Regius Professor of Physic, University of Cambridge. 222 pp., 8 plates, $4.50. Philadelphia and Toronto: The Blakiston Company, 1948.

This book gives a brief description of the more important diseases of the pituitary, pineal gland, thyroid, parathyroid, thymus, adrenal glands, ovaries, testes, and the breast. In each chapter there is a short discussion of the anatomy, histology, and physiology followed by a concise presentation of the more important diseases of these glands. In general there has been an oversimplification of the entire subject. The book will be of little value for the average pathologist.

San Francisco GERSON R. BISKIND

General Endocrinology. C. DONNELL TURNER, PH.D., Associate Professor of Zoology, Northwestern University. 604 pp., 164 figs., $6.75. Philadelphia and London: W. B. Saunders Company, 1948.

The increasing emphasis that is being placed on normal and altered physiologic activity of the endocrine glands makes it imperative that the pathologist have an understanding of the basic relationships of these glands to each other as well as to the chemical and physiologic processes of the body in order to interpret many pathologic conditions. In this book, the fundamentals of endocrinology are clearly described by a careful, concise exposition of the important anatomical and physiologic facts. Each subject is developed by an evaluation of the pertinent experiments and principles and, in general, the author has selected his material wisely. Despite the fact that he has intended the book primarily for beginning students who are concentrating on experimental biology, it is recommended to pathologists as a reference book for endocrine problems.

GERSON R. BISKIND

Hematology For Students and Practitioners. Revised Ed. 2. By WILLIS M. FOWLER, M.D., Professor of Internal Medicine, University of Iowa, Iowa City; with a chapter by ELMER L. DEGOWIN, M.D., Associate Professor of Internal Medicine, University of Iowa, Iowa City. 535 pp., 184 figs., 8 color plates, $8.50. New York: Paul B. Hoeber, Inc., 1949.

The first edition of this book, published in 1945, was reasonably designed to fill the needs principally of the medical student. Now the revised second edition brings the subject up to date concisely by avoiding prolonged discussion of controversial material. The text is not designed to serve as a reference for the hematologist or the histologist. Written from an internist's point-of-view, hematologic aberrations are admirably integrated with the whole clinical picture in each disease.

The chapter in the first edition entitled The Blood Picture in Certain Infections, which was principally concerned with parasitic and tropical diseases, has been omitted from the second edition. Discussion has been broadened generally, and particularly with reference to the sections on multiple myeloma, infectious mononucleosis and the hemoglobinurias. Newer therapeutic agents are reviewed, particularly folic acid, urethane, nitrogen mustard and radio-active phosphorous. The excellent chapter on Transfusion of Whole Blood and Blood Derivatives by Dr. DeGowin has been expanded and includes current terminology in the field of the Rh factors.

Unchanged is the author's selection of the Sabin-Doan interpretation of megaloblasts which is rather arbitrarily stated as being most widely accepted. The reviewer similarly feels that too much emphasis is given to the Banti syndrome, where a revised discussion of the concept of "hypersplenism" would be in order. The color plates are unchanged, some of them being rather diagrammatic. There are a few typographical errors, particularly in the references.

Despite these criticisms, the reviewer believes that the author has succeeded in his purpose of producing a text that is well adapted to the needs of medical students and of all practitioners of medicine who do not limit themselves to hematology or histopathology.

Eloise, Michigan JOHN B. MOYER

BOOKS RECEIVED

Abramson, H. A.: Psychodynamics and the Allergic Patient. 81 pp., 2 figs. St. Paul and Minneapolis, Minn.: The Bruce Publishing Company, 1948.

Arquivos do Instituto de Biologia do Exército. Ano 8, Numero 8, 1947. Rio de Janeiro: I.B.E.—Rua Licinio Cardoso.

Barnard, C. I.: The Rockefeller Foundation, A Review for 1948. 71 pp., illust. New York: The Rockefeller Foundation, 1949.

Boell, E. J., Costello, D. P., Detwiler, S. R., Fankhauser, G., Holtfreter, J., Nicholas, F. S., Rose, S. M., Rudnick, D., and Stone, L. S.: Recent studies in the mechanisms of embryonic development. 206 pp., illust. $3.50. New York: The New York Academy of Sciences, 1948.

Brams, W. A.: Treatment of Heart Disease. 195 pp., 11 figs. $3.50. Philadelphia: W. B. Saunders Company, 1948.

Kneucker, A. W.: Richtleinen Einer Philosophie der Medizin. 197 pp. $4.50. Vienna: William Maudrich, Publisher, 1948.

Kruse, H. D.: A Concept of the Etiological Complex of Deficiency States With Especial Consideration of Conditions. Reprinted from the Milbank Memorial Fund Quarterly, Vol. XXVII. No. 1, Jan., 1949. New York: Milbank Memorial Fund, 1949.

Leriche, R.: Anévrysmes Artériels et Fistules Artério-Veineuses. 314 pp., 26 figs. 900 francs. Paris: Masson et Cie, 1949.

Manual de Enfermedades Infecciosas. Vol. II. 297 pp. 20 pesetas. Barcelona: Coleccion Espanola de Monografias Medicas, 1948.

Manual de Enfermedades Infessiosas. Vol. III. 331 pp. Barcelona: Coleccion Espanola de Monografias Medicas, 1948.

Nonidez, J. F., and Windle, W. F.: Textbook of Histology. 456 pp., 287 figs. $6.75. New York: McGraw-Hill Book Company, Inc., 1949.

Pepper, O. H. P.: Medical Etymology. 263 pp. $5.00. Philadelphia: W. B. Saunders Company, 1949.

Potter, V. R.: Methods in Medical Research. Vol. I. 372 pp., illust. $8.00. Chicago: Year Book Publishers, Inc., 1948.

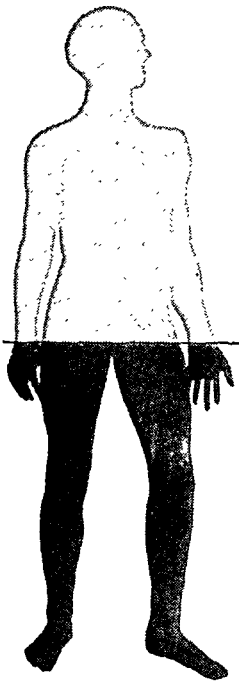
Sigler, L. H.: Cardiovascular Disease; Fundamental, Differential Diagnosis, Prognosis and Treatment. 551 pp., 149 figs. $10.00. New York: Grune and Stratton, 1949.

The New York Academy of Sciences:

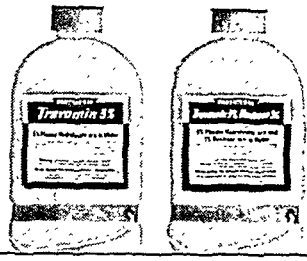
Antibiotics Derived from *Bacillus polymyxa*. Vol. 51, Art. 5. 160 pp., illust. $2.25. New York Academy of Sciences, 1949.

Symposium: Amino Acid Excretion. Vol. 10, Art. 8. 36 pp., illust. New York: The New York Academy of Sciences, 1949.

The Adrenal Cortex. Vol. 50, Art. 6. 170 pp., illust. $3.00. New York: The New York Academy of Sciences, 1949.



a valuable aid



in postsurgical recovery

A large percentage of patients enter surgery at a low plasma protein level, and the operative procedure makes further inroads on the already depleted protein reserves.[1,2] A lowered protein level is unfavorable to recovery. It predisposes the patient to pulmonary edema and infection, retarded wound and fracture healing and impaired liver function.[3,4] A high protein level is conducive to rapid healing.[5]

Since diets immediately following surgery are usually inadequate, protein digests given intravenously result in improved strength, appetite, and wound healing. Consequently, they greatly accelerate recovery.[6,7]

2000 cc. of TRAVAMIN 5% a day will satisfy the protein requirements of a high percentage of surgical patients. 2000 to 4000 cc. a day are given according to requirements.[8] TRAVAMIN is made from bovine plasma.

TRAVAMIN 5% IN WATER
TRAVAMIN 5%, DEXTROSE 5% IN WATER

bibliography

1. WHITE, C. S.: "Intravenous Fluids with Particular Reference to Amino Acids," M. Ann. District of Columbia, 15:251 (June) 1946

2. MEYER, J. A., KOZOLL, D. D.: "Protein Deficiency in Surgical Patients," Surg., Gyn. & Obst., 78:181 (February) 1944

3. GLENN, F., *et al:* "Use of Protein Hydrolysates —Conference on Therapy," Am. J. Med., 3:472 (October) 1947

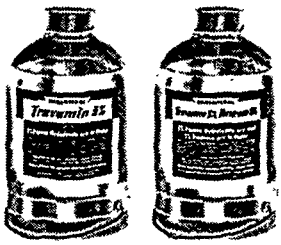
4. CASTEN, D., BODENHEIMER, M., BARCHAM, I.: "A Study of Plasma Protein Variations in Surgical Patients," Ann. Surg., 117:52 (January) 1943

5. BAILEY, H.: Surgery of Modern Warfare, Baltimore, Williams and Willkins Co., 1941, p. 847

6. DAVIS, H. H.: "Amino Acid Intravenously in Surgical Patients," Nebraska Med. Journal, 30:51 (February) 1945

7. CANNON, P. R.: "Protein Metabolism and Resistance to Infection," J. Mich. M. Soc., 43:323 (April) 1944

8. WEINSTEIN, J. J.: "Intravenous, Subcutaneous and Rapid Intramuscular Infusions of 'Protein Hydrolysate'," Surg., Gyn. & Obst., 87:93 (July) 1948



PARENTERAL **Travamin*** 5% PLASMA HYDROLYSATE

* *formerly PROTEIN HYDROLYSATE, BAXTER*

Product of
BAXTER LABORATORIES, INC.
Morton Grove, Illinois

Distributed and available only in the 37 states east of the Rockies (except in the city of El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION

GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

MEMBERS OF

STATE SOCIETIES OF

CLINICAL

PATHOLOGISTS

You can lend valuable aid and moral support to the registered medical technologists of your state.

Help them with their local and state programs and, more particularly, with their organizational problems.

The problems of the medical technologists manifestly are the concern of the pathologists, because the two professions are so closely linked together.

REGISTRY OF
MEDICAL TECHNOLOGISTS
of the
American Society of Clinical Pathologists
Muncie, Indiana

Advertisers
in the Journal
Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.

BBL

MEDIA FOR
ENTERIC PATHOGENS

D. C. L. S. AGAR
for *isolation* of Salmonella, Shigella and Eberthella

T. S. I. AGAR
for *differentiation* of Coliform organisms, Proteus, Salmonella and Shigella and for use in *typing* when agglutination is carried out.

TRYPTICASE AGAR BASE
for determination of *motility*, for *maintenance* and for further *fermentation* studies, if necessary

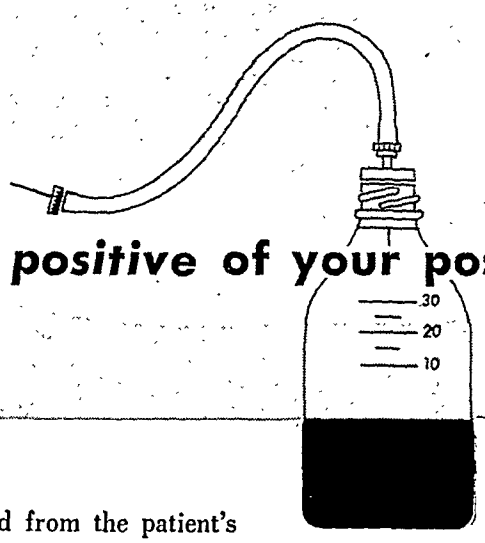
UREASE TEST MEDIUM
for differentiation of *Proteus* by utilization of urea

PHENOL RED BROTH
for *fermentation* studies, when fluid media are preferred

Prices and literature sent on request

BALTIMORE
BIOLOGICAL LABORATORY
Baltimore, Md.

NOW



you can be *positive* of your positives

Direct delivery of blood from the patient's vein, through a closed, sterilized system, into Trypticase Soy Broth media in the new B-D VACUTAINER CULTURE BOTTLE . . .

- **Provides *positive* positives and valid negatives**
- **Prevents outside contamination**
- **Avoids incorrect selection and multiplicity of media**

Aerobic or anaerobic conditions may be obtained at time blood specimen is taken. Bottle reaches laboratory ready for immediate incubation . . . speeds laboratory handling . . . provides faster diagnosis. Microaerophiles grow luxuriantly.

Write for descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE and learn how easy it is to add this new element of assurance to your blood culture procedures.

with the

B-D

VACUTAINER®

CULTURE

BOTTLE

containing Trypticase Soy Broth with CO_2 added

Prepared and sterilized by Baltimore Biological Laboratories, Inc., Baltimore, Md.

BECTON, DICKINSON & CO., Dept. 27-K
15 Ames Avenue,
Rutherford, N. J.

Please send me descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE that takes the guesswork out of blood culture procedures.

NAME_____

ADDRESS_____

CITY_____ ZONE____ STATE_____

BECTON, DICKINSON & CO., RUTHERFORD, N. J.

MEDICAL CLASSICS

SMITH AND GAULT

Essentials of Pathology

(3rd Edition)

"There is no more suitable text available to the medical student than this interesting, informative and correlative introduction to the essentials of pathology." An "excellent presentation of case histories, textual material and illustrations," reports the *American Journal of Clinical Pathology*. New material has been added, case histories condensed, repetitious material reduced. The ideal book on the subject.

740 Illus., 764 Pages, $12.00

STITT, CLOUGH & BRANHAM

Practical Bacteriology, Hematology & Parasitology

This is a world-famous book, now in its 10th edition. Up-to-date and valuable additions to material, including many new subjects and sections, have been contributed by specialists in their fields; for instance, the new method of preparing colloidal gold solutions now in use at the Naval Medical School Laboratories. There is a summary of the present status of antibiotics . . . and many other informative features. Many new illustrations. The *Review of Gastroenterology* calls it "An indispensable volume for the pathologist and the diagnostician." Order a copy of this classic for personal examination.

765 Illus., 991 Pages, $10.00

Blakiston's New Gould is the only completely new medical dictionary in 38 years. Do you have one? Textbook edition, $8.50—Thin paper edition, $10.75—Deluxe edition, $3.50.

THE BLAKISTON COMPANY

1012 Walnut Street, Philadelphia 5, Pa.

Please send the items checked: my check is enclosed; charge my account. It is understood that you will credit my account in full for any books that I wish to return within 10 days.

☐ Smith and Gault's Essentials of Pathology

☐ Stitt, Clough & Branham's Practical Bacteriology, Hematology and Parasitology

☐ Dictionary

NAME..... ADDRESS.....

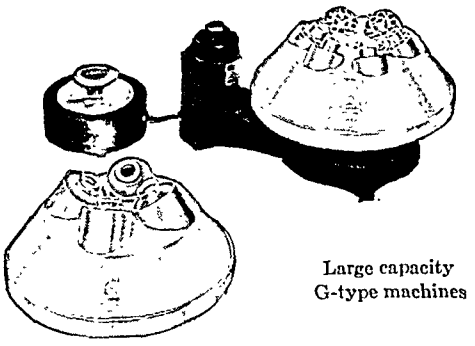
CITY..... ZONE..... STATE.....

AJCP 10-49

SERVALL

ANGLE CENTRIFUGES

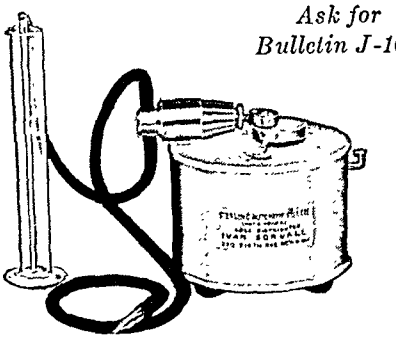
Ask for Bulletin J-101 with complete data on all models



Large capacity G-type machines

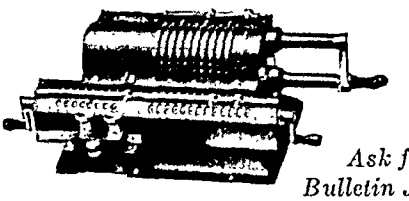
Type G/1, for 6 bottles of 250 cc each
18 tubes of 60 cc each
42 tubes of 15 cc each
78 tubes of 10 cc each
Type G/2, for 4 bottles of 500 cc each
Type G/3, for 10 tubes of 100 cc each

Ask for Bulletin J-102



STERLING AUTOMATIC AND HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc. Easy to operate—Time-Saving



Ask for Bulletin J-103

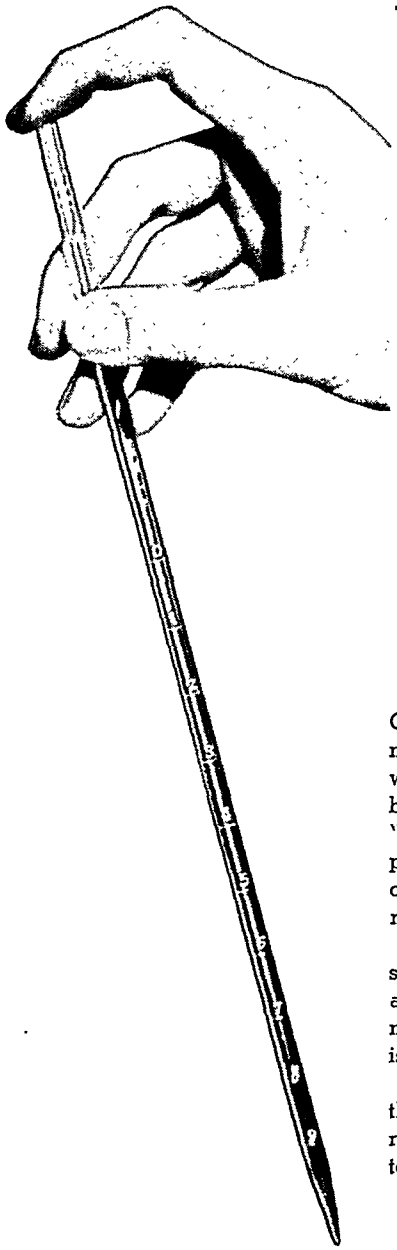
ORIGINAL—ODHNER

Portable Calculator for the Scientist
Many different models
Efficient - Sturdy - Low Priced

Manufacturers and Distributors:

IVAN SORVALL, Inc.

210 FIFTH AVE.-NEW YORK 10, N. Y.



T.M. REG. PYREX U.S. PAT. OFF. BRAND

PIPETTES...

Quickly readable, unaffected by repeated sterilization

Clinical research makes many demands on the technicians in charge. Speed is one of them. But speed without accuracy avails nothing. That's why PYREX brand Pipettes are designed for easy readability. The "Lifetime Red" graduations are permanent, part of the pipette body itself. "Lifetime Red" will not wear or chip off in long service. Tips are fabricated to give maximum protection from chipping.

Made from PYREX brand glass No. 7740, they withstand repeated sterilization (wet or dry). They are unaffected thermally or chemically by any of the methods normally employed in laboratories where sterilization is a major factor in routine or in specialized work.

The measuring and serological types more than meet the tolerances of Federal Specification D.D. V-581 in regard to accuracy limits. When you call your laboratory supply dealer, ask for PYREX brand Pipettes!

CORNING means research in GLASS

T.M. REG. PYREX U.S. PAT. OFF.

Stocked by Leading Laboratory Supply Houses

CORNING GLASS WORKS • CORNING, N. Y.

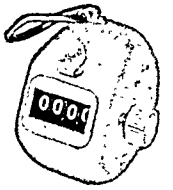
LABORATORY GLASSWARE

TECHNICAL PRODUCTS DIVISION: LABORATORY GLASSWARE • SIGNALWARE • GLASS PIPE • GAUGE GLASSES • LIGHTINGWARE • OPTICAL GLASS • GLASS COMPONENTS

Veeder-Root Hand Tallies for Blood Count



These quick, accurate instruments, used in blood analysis, are available in two models, the double and single, both used for differential and red and white count. When held cupped in the hand a slight pressure on operating lever registers one additional count. When the totals have been recorded, the figures are quickly returned to zero by turning the reset knob. Used in either hand, the other is left free for adjusting the microscope or tabulating. If not available from your Medical Supply House, order direct using the handy coupon below.



Veeder-Root

World's Largest Manufacturers of Counting Devices

VEEDER-ROOT, INC., HARTFORD 2, CONN. A

Please send the following:

☐ Veeder-Root Double Hand Tally Counters (@ $17.00 each)

☐ Veeder-Root Single Hand Tally Counters (@ $7.50 each)

☐ Descriptive folder on Hand Tally Counters

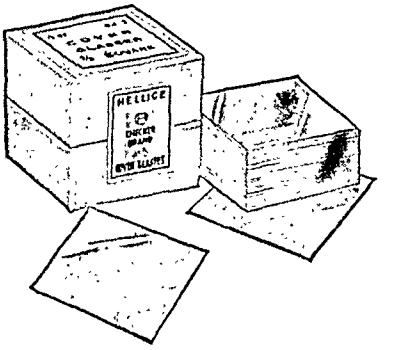
Institution.....

Street.....*City*.....*State*.....

Name.....

HELLIGE CHECKER BRAND MICRO COVER GLASSES

Again Available!



We have resumed the production of micro cover glasses, discontinued during the war because our efforts were directed primarily toward manufacturing for the U.S. Army and Navy Hellige specialties not obtainable elsewhere.

Now we are able to offer again the same surpassing quality which made Hellige Micro Cover Glasses the choice of the medical profession for more than half a century.

Write Today for our Attractive Prices

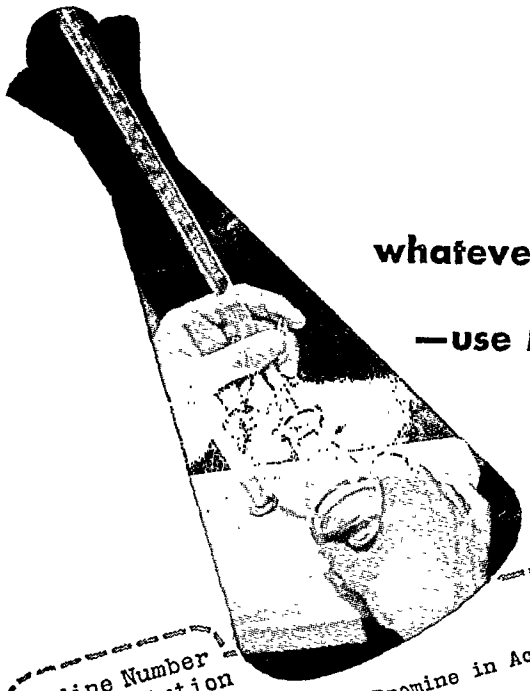
HELLIGE

INCORPORATED

3718 NORTHERN BLVD.

LONG ISLAND CITY 1, N.Y.

MALLINCKRODT



whatever you do

—use Mallinckrodt...

Use M.C.W. only!

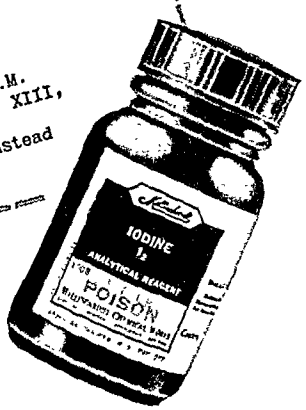
Iodine Number
Hanus Solution

Solution of Iodine and Bromine in Acetic Acid.

Requires:
Iodine, A.R.
Glacial Acetic Acid, A.R.—dichromate tested.
Potassium Iodide, A.R.
Sodium Thiosulfate, A.R.
Bromine, A.R.

Standard Method in A.O.A.C. methods (1945) p. 494 (high precision). Also A.S.T.M. See also Snell and Biffen, p. 719; U.S.P. XIII, p. 839; Furman-Scott, p. 1800.

Prefer this to Wij's solution (uses Cl instead of Br).



But whichever directions you choose, use Mallinckrodt Analytical Reagents. They are consistently the fine reagents A.C.S. specifications require. When your reagents bear the Mallinckrodt label you are sure of uniform dependable purity, and exceptional performance in every test.

ALWAYS SPECIFY MALLINCKRODT REAGENTS IN ORIGINAL PACKAGES

82 Years of Service to Chemical Users

MALLINCKRODT CHEMICAL WORKS

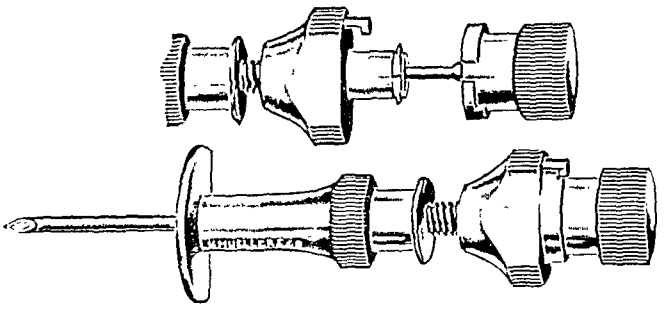
MALLINCKRODT STREET, ST. LOUIS 7, MO. • 72 GOLD STREET, NEW YORK 8, N.Y.
CHICAGO • CINCINNATI • CLEVELAND • LOS ANGELES • MONTREAL • PHILADELPHIA • SAN FRANCISCO

UNIFORM DEPENDABLE PURITY

The New, Improved

UNIVERSITY OF ILLINOIS STERNAL PUNCTURE NEEDLE

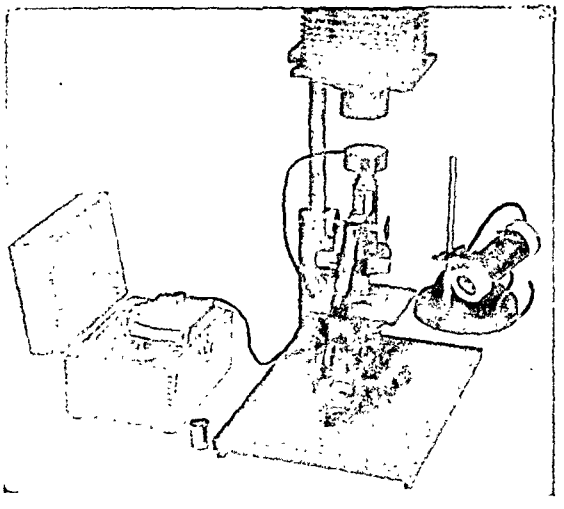
As Modified by Louis R. Limarzi, M.D. and Paul L. Bedinger, M.D., Chicago



Easier for both patient and operator, this new sternal puncture needle is also useful in punctures of vertebral spine, femur or tibia. Its special lock prevents displacing obturator when entering any type of bone. Tightly-fitted stylet prevents procaine, peripheral blood, spicules of bone marrow or clotted blood from entering lumen of the needle. Needle has a Luer Lok hub, and aspiration or infusion technique may be used, if desired. Rustless. Specify TH-251. Each, *$13.50.*

Order direct from

V. MUELLER & CO. 408 S. HONORE ST. CHICAGO 12, ILLINOIS



PHOTOVOLT

Exposure Photometer Mod. 200-M

for

PHOTOMICROGRAPHY

A photoelectric instrument of high accuracy and sensitivity, dependable and simple in operation, designed to measure light at microscope exit for determination of exact exposure time in black-and-white as well as color photomicrography $65.—

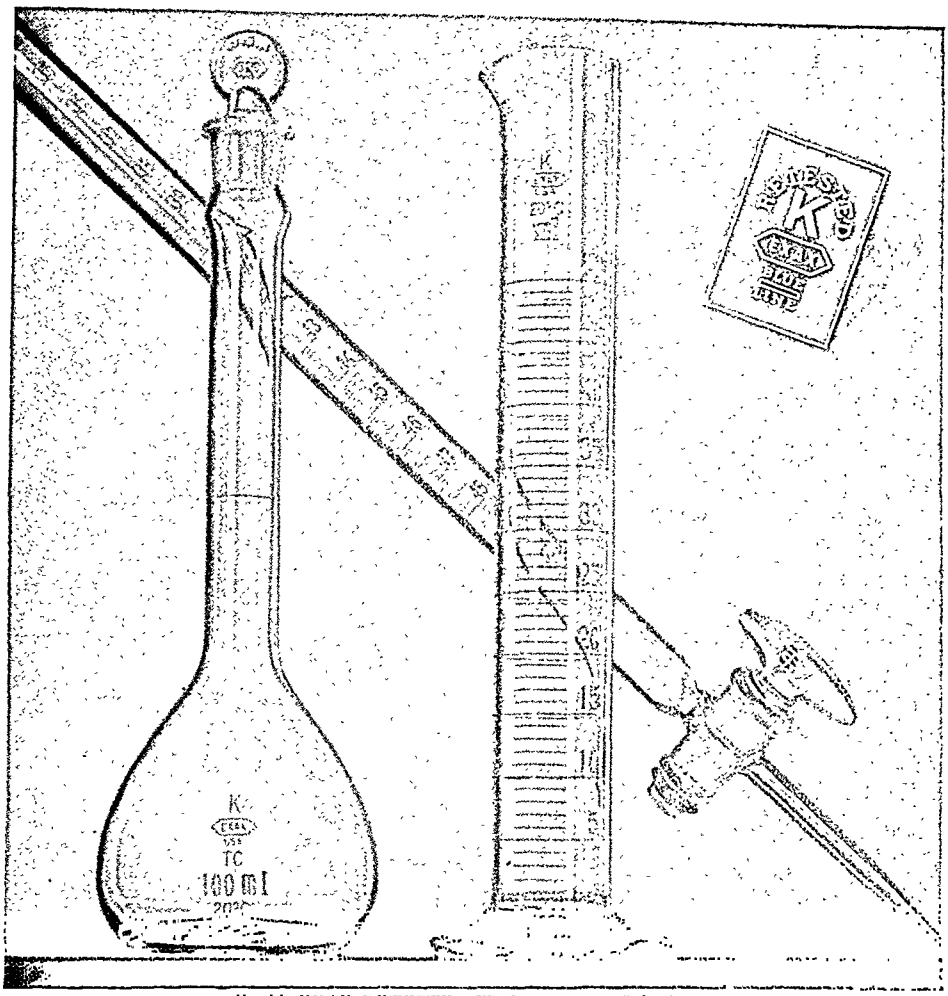
Also: **Electronic Photometers Mod. 500-M and 512-M**

for ❶light and color readings on small areas in focal plane
❷quantitative histochemical and cytochemical analysis
❸measurement of lowest light values with photo-multiplier

Write for Circular #810 and for 22-page Technical Information Bulletin #812

95 Madison Ave. **PHOTOVOLT CORP.** New York 16, N. Y.

Clinical Colorimeters, Fluorescence Meters, Hemoglobinometers, pH Meters, Densitometers



Kimble EXAX RETESTED Flask No. 25015; Cylinder No. 20030; Burette No. 17030.

There's no substitute for EXAX RETESTED quality

EXAX RETESTED is America's No. 1 choice for accurate laboratory determinations simply because it is made and intended for that purpose. EXAX won't bounce, but it is thoroughly annealed for toughness . . . will stand everyday handling and usage.

The great value of EXAX RETESTED lies in certain exclusive advantages. It is a special formula of glass, precision-made by specialty craftsmen.

The fine deep graduations are of a type which readily accepts the permanent fused-in blue filler. *And* . . . all calibrations are marked and checked and *retested* with standards of highest precision.

So remember, if accuracy and fitness for the job are important, there is no substitute for EXAX RETESTED glassware.

Look for the Kimble "K", the visible guarantee of invisible quality.

KIMBLE GLASS TOLEDO 1, OHIO

Division of Owens-Illinois Glass Company

LABORATORY TECHNIQUE

In Biology and Medicine

SECOND EDITION

By E. V. Cowdry

Professor of Anatomy, Washington University

An excellent laboratory instrument for the technician

- Expanded to include related physical methods and micro-chemical technique.
- New methods and improvements in standard techniques added.
- A laboratory tool that broadens the utility of every other piece of laboratory equipment in its scope.
- It is "definite information on specific matters," as the author states.
- And the definite information is really accessible—all alphabetically arranged under names of structures, and strains, and elements, and names of procedures. You can hardly miss.
- Thoroughly documented with the references where you want them—as part of the text you are reading at the moment.
- In short, as the *American Journal of Medical Technology* puts it, the worker "will find it useful at the work table instead of on the book shelf."

LABORATORY TECHNIQUE

Cloth 6 x 9 **224 pages** **self-indexing** **$4.00**

THE WILLIAMS & WILKINS COMPANY

Mt. Royal & Guilford Aves.

Baltimore 2, Maryland

for

BLOOD TYPING
RH TESTING

you need the highest-titred serum available.

ANTI-HUMAN PRECIPITIN SERUM (rabbit)
for the Coombs Test

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human Red Cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic Serum
for control tests

P.S.A.
(Paraffin Section Adhesive)

Write for complete information.

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York

A POSTGRADUATE COURSE IN

CLINICAL PATHOLOGY

FOR

LABORATORY TECHNICIANS

December 1-3, 1949

Offered By

WASHINGTON UNIVERSITY

SCHOOL OF MEDICINE

Tuition $25.00

The course is designed as a period of postgraduate training for laboratory technicians. Recent advances in clinical pathology will be reviewed.

For further information write:

Director, Division of Postgraduate Studies

Washington University School of Medicine
4580 Scott Avenue
St. Louis 10, Missouri

ALBIMI

PREPARED
CULTURE MEDIA

In screw-capped one ounce bottles providing a large surface area

LOEFFLER'S COAGULATED BLOOD SERUM

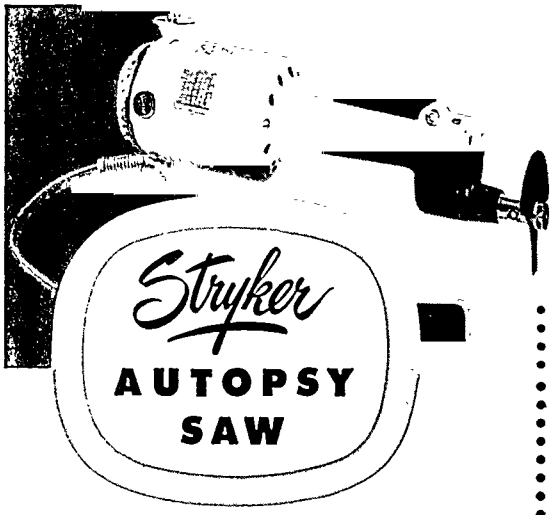
LÖWENSTEIN'S MEDIUM
For tubercle bacilli

PETRAGNANI'S MEDIUM
For tubercle bacilli

Other prepared media available
Literature on request

ALBIMI LABORATORIES, Inc.

16 CLINTON STREET
BROOKLYN 2, N. Y.



A new instrument which simplifies bone cutting

Electrically driven, oscillates at high speed to cut bone efficiently with complete safety. Cutting blades do not hurl material. Two-sided blade can be adjusted to three positions. Blade, arbor and shaft are stainless steel. Write Department C for complete information.

ORTHOPEDIC FRAME COMPANY Kalamazoo Michigan

REDUCTION!

Effective September 1st, 1949

STANDARDIZED
DESICCATED COMPLEMENT

3 cc	$2.00
5 cc	3.00
7 cc	4.00
15 cc	7.50

(*10% discount on lots of 50 or more*)

All reagents for the Kolmer-Wassermann Test, produced *by Cappel*, are standardized against each other. For this reason we recommend the use of the following reagents:

KOLMER-WASSERMANN ANTIGEN
(Standard or New)

ANTI-SHEEP HEMOLYSIN
(Glycerinated or Desiccated)

SHEEP CELLS
can be prepared from one of the following:

STERILE SHEEP BLOOD (Alsever's)

STERILE SHEEP BLOOD (Citrated)

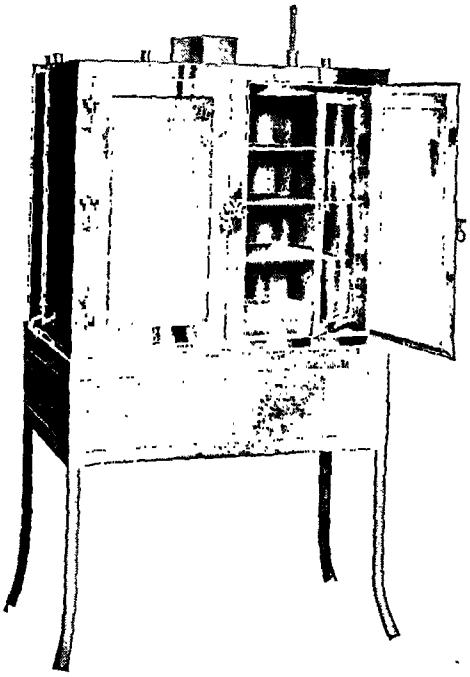
or

stabilized 10% suspension
WASHED POOLED SHEEP CELLS

CAPPEL LABORATORIES
Diagnostic Reagents

Box 352 Wayne, Pa.

NOW! MONEL METAL adds Years to the Service of Castle INCUBATORS



Castle's proved performance now has a new advantage in construction—Monel throughout.

This lifetime metal eliminates pitting and porosity that often appear in copper (formerly the standard metal in incubators) from the action of chemicals now found in water systems.

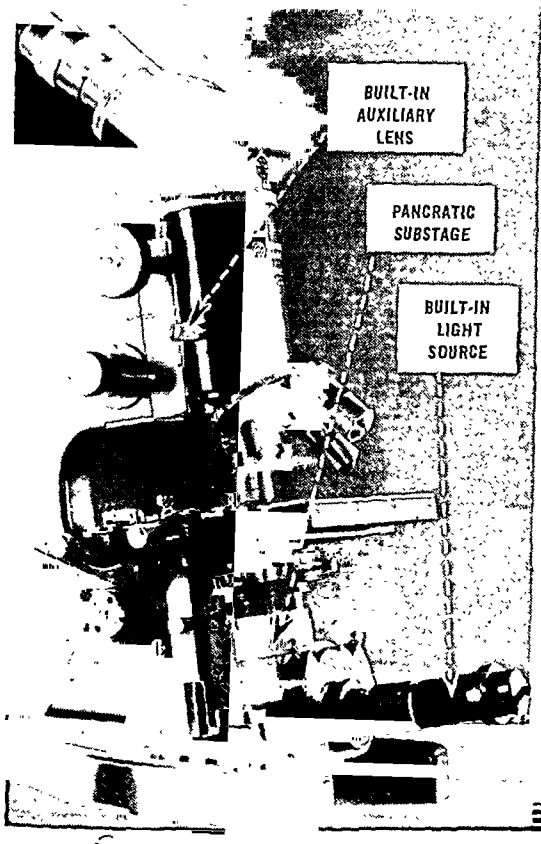
Precise performance is essential for bacteriological work . . . and precision is the outstanding feature of Castle Incubators:

CONSTANT TEMPERATURE, with ¼° to ½°C. ± variation during the incubating period, despite changes in room temperature;

UNIFORM TEMPERATURE, with no more than ⅞°C. ± variation between top and bottom of the chamber *when loaded.*

For full details, see your Castle dealer, or write: Wilmot Castle Co., 1216 University Ave., Rochester 7, New York.

Castle BACTERIOLOGICAL APPARATUS



GALILEO CORP. OF AMERICA, Inc.

Announces

The VNP Phase-Contrast Microscope with features which open completely new possibilities in phase-contrast technique.

•

For descriptive brochure write

PFALTZ & BAUER, Inc.

Empire State Building, New York 1, N. Y.

Sole agents for

GALILEO CORPORATION OF AMERICA, Inc.

745 Fifth Avenue, New York 22, N. Y.

Distributor for Officine Galileo, Italy

Florence • Milan • Venice

Since 1881 manufacturers of research microscopes and microscopical equipment of all kinds, refractometers, polarimeters, and analytical balances.

Aid to Early Diagnosis

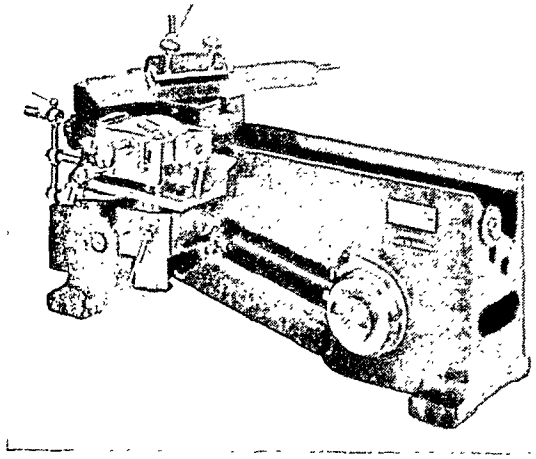
BRONCHIOGENIC CARCINOMA and ADENOMA

By B. M. FRIED, M.D.
Associate Attending Physician, Montefiore Hospital, New York

Dr. Fried's book is based on extensive studies of large clinical and post mortem material. It covers both pathological and clinical aspects of all types of bronchiogenic and mediastinal tumors. Throughout the text great emphasis is placed on the vital importance of early diagnosis. To this end, all the modern diagnostic methods at the disposal of the physician are discussed, as well as etiology and treatment. Both primary and metastatic carcinoma are covered, and also bronchiogenic adenoma and tumors of the mediastinum. 326 pp. 118 figs. $6.00

THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves. Baltimore 2, Md.

REICHERT MICROTOMES
of Vienna



Model "OmE" Sliding Type, 40 cm
Section Thicknesses from 1 to 30 Microns Complete with Automatic Object Feed, Object Clamp and Knife $545.00
Model "OmP" Freezing Microtome complete with knife $235.00

Write for literature—Immediate Delivery

LABORATORY EQUIPMENT CO.
118-11th St., San Francisco, Calif.

Prepared Clinical Laboratory Reagents for

BIOCHEMICAL
BACTERIOLOGICAL
HISTOLOGICAL
SEROLOGICAL
HEMATOLOGICAL
PROCEDURES

Prepared Bacteriological Culture Media in Sterile Screw Cap Test Tubes—
Petragnani T.B. Medium
One Dozen 2.75
Loeffler's Blood Serum
One Dozen 2.25

HEMATOLOGICAL AND BACTERIOLOGICAL STAINS

CATALOG UPON REQUEST

LABORATORY REAGENTS COMPANY
Woodbridge, New Jersey

(In writing to advertisers, please mention the Journal—it helps.)

WHY YOU SHOULD SPECIFY

"VACSEAL"

GUINEA-PIG COMPLEMENT

Because it is a superior complement—the result of a decade of experience in guinea-pig complement production, the proper choice and preparation of animals and the use of improved technics.

Because our large output enables us to pool the serum of no less than 100 guinea pigs per lot, assuring a more uniform titer. It also makes it possible for you to obtain a complete lot of any complement which has proved, through pretesting, to be particularly desirable for your purpose.

Because of the high vacuum and all-glass containers, storage for at least two years is possible. To you this means economy and convenience.

"Vacseal" Complement is distributed in three sizes with a special diluent for restoration to the following amounts:

3 ml. 7 ml. 20 ml.

Any recognized laboratory will receive a trial 3 ml. sample upon request.

CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK

Since 1928

GUINEA PIGS

RABBITS

(Friedman Does)

WHITE MICE

Satisfactorily shipped 1000 miles and more

By

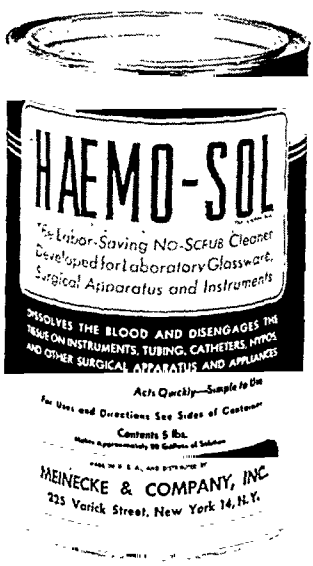
HILLTOP CAVIARY

Box 195 Scottdale, Pa.

Phone 8113R12

★

When writing for quotations or other information please indicate weight, sex and NUMBER of animals you require.



Literature and samples on request. Write us regarding your specific cleaning problems.

NO RESIDUE

TO INTERFERE WITH YOUR EXACTING TITRATIONS AND TESTS

Good technique demands that laboratory glassware be not merely washed but *thoroughly cleansed.* This you can be sure of when you use HAEMO-SOL, the original chemically prepared cleaning agent and blood solvent, specifically developed for laboratory and hospital work.

Of equal importance, laboratory glassware must be free of even the slightest trace of detergent-induced deposit or residue. HAEMO-SOL affords you this protection due to its extraordinary solubility and free rinsing qualities, indicated by the fact that it dissolves to a crystal-clear solution evincing no turbidity or precipitate on standing. Furthermore, HAEMO-SOL'S non-etching effect on glass, correlated with its low pH, saves costly replacement of laboratory apparatus.

MEINECKE & COMPANY, INC.
225 VARICK STREET • NEW YORK 14, N. Y.

A NEW PERIODICAL

ANGIOLOGY

The Journal of Vascular Diseases

Edited by SAUL SAMUELS, M.D., New York City
Assisted by an International Editorial Board

The growing interest in peripheral vascular diseases and the increase in research and clinical work has prompted the establishment of a periodical to be devoted exclusively to this field.

A distinguished group of clinicians from the United States and the principal foreign countries is actively promoting the new journal and the first issue will appear in February 1950. One volume of 600 pages will be published each year and issues will appear in February, April, June, August, October and December.

Be a charter subscriber by mailing your subscription order now directly to the publishers.

Subscription price $8.00

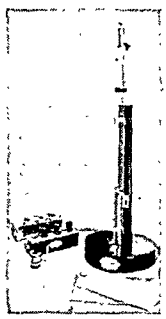
THE WILLIAMS & WILKINS CO.
Baltimore 2, Maryland

(In writing to advertisers, please mention the Journal—it helps.)

The New and Improved LaMotte Falling-Drop Densiometer

Two Models

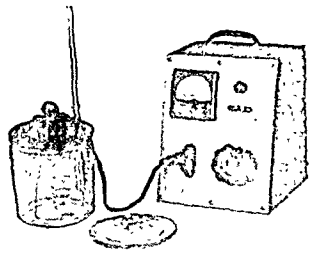
An Important Adjunct in Treating Cases of Shock



For determining blood proteins and specific gravity of body fluids. These units were developed exclusively with the cooperation of Dr. W. F. Hamilton and the late Dr. H. G. Barbour, and were approved by them.
The instrument, designed for determining Total Proteins and the Specific Gravity of Body Fluids, is available in large and small models. Send for complete information.

If you do not have the LaMotte Blood Chemistry Handbook, a complimentary copy will be sent upon request without obligation.

LaMotte Chemical Products Co.
Dept. D Towson 4, Maryland

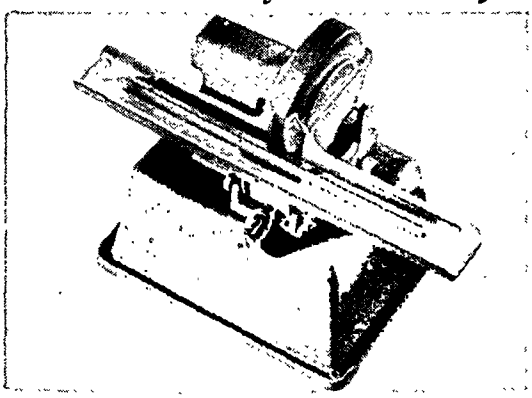


Eberbach BONE DECALCIFIER

Rapidly decalcifies bone for histologic section. Power unit supplies DC to electrolytic cell. Preserves soft tissue patterns and staining properties better than acid immersion methods and reduces time required from days to hours. Write for Bulletin 180 and operational data.

Eberbach & Son Company
Ann Arbor, Mich.
Laboratory Apparatus & Supplies

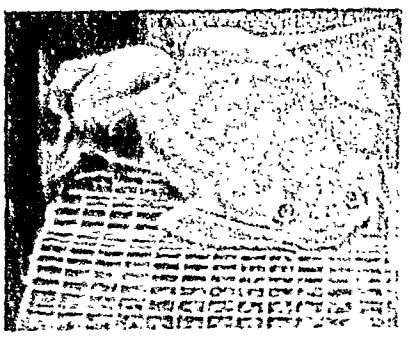
INDISPENSABLE
in your laboratory...



the Berkeley MICROTOME KNIFE SHARPENER
for perfect edges...three times faster

Operation is simple, eliminates honing and stropping. Assures hollow-ground edges that cut better and last much longer. *Send for our Illustrated Bulletin MKS-14*

Berkeley Scientific Company
SIXTH AND NEVIN AVENUE • RICHMOND, CALIF.



XENOPUS LAEVIS FROG

Pregnancy Diagnosis
Animal Costs Greatly Reduced

We assure you of a continuous supply. Accurate, speedy and inexpensive. Average animal cost per test, 20 cents to 35 cents. We carry a complete stock of Laboratory Tanks, Isolation Jars, Holders, and correct reagents and apparatus for running tests Speedy delivery via AIR EXPRESS. Current brochure showing new low prices sent upon request.

Jay E. Cook, Importer

P. O. Box 302 Baltimore 3, Md., U.S.A.
Cable Address—XENOPUS-BALTIMORE

(In writing to advertisers, please mention the Journal—it helps.)

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia...O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Benbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

American Society of Clinical Pathologists

OFFICERS

President: Dr. O. A. Brines, Wayne University College of Medicine, Detroit 26, Michigan
Vice-President: Dr. C. H. Manlove, 5725 S.E. Stark Street, Portland 15, Oregon
President-Elect: Dr. J. B. McNaught, University of Colorado School of Medicine, Denver, Colorado
Secretary-Treasurer: Dr. C. G. Culbertson, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana

STANDING COMMITTEES

EXECUTIVE COMMITTEE

W. S. Thomas, Chairman, Rochester, N. Y.
T. J. Curphey, Long Island, N. Y.
F. W. Konzelmann, Atlantic City, N. J.
L. W. Larson, Bismarck, N. D.
S. P. Reimann, Philadelphia, Pa.
J. R. Schenken, Omaha, Neb.

BOARD OF CENSORS

L. W. Diggs, Chairman, Memphis, Tenn.
J. J. Andujar, Fort Worth, Tex.
F. C. Coleman, Des Moines, Iowa
J. W. Howard, Wilmington, Del.
V. M. Johnson, West Palm Beach, Fla.
C. P. Larson, Tacoma, Wash.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. Montgomery, Chairman, Muncie, Ind.
A. G. Foord, Pasadena, Cal.
F. B. Lynch, Jr., Philadelphia, Pa.
F. B. Queen, Portland, Ore.
R. G. Stillman, New York, N. Y.
A. H. Wells, Duluth, Minn.

APPOINTED COMMITTEES 1948-1949

GENERAL RESEARCH COMMITTEE

M. Strumia, Chairman
B. S. Kline
A. H. Sanford
W. M. Simpson

COMMITTEE ON HEMATOLOGIC TERMINOLOGY

E. E. Osgood, Chairman
L. Berman
R. R. Kracke
F. J. Heck
L. R. Limarzi

BLOOD BANK COMMITTEE

D. H. Kaump, Chairman
L. Y. Dyrenforth
L. W. Larson
F. W. Hartman
R. G. Stillman

COMMITTEE ON REGISTRY OF GENERAL TUMORS

J. W. Kernohan, Chairman
L. V. Ackerman
Col. J. E. Ash
W. A. D. Anderson
S. W. Lippincott
I. Perry

REPRESENTATIVE ON NATIONAL RESEARCH COUNCIL SUBCOMMITTEE ON ONCOLOGY

S. Warren

REPRESENTATIVE ON BOARD OF GOVERNORS OF COLLEGE OF AMERICAN PATHOLOGISTS

T. J. Curphey

COMMITTEE ON SEMINAR

I. Davidsohn, Chairman
P. R. Cannon
J. B. McNaught
Gen. R. O. Dart
R. A. Moore

PROGRAM COMMITTEE

C. G. Culbertson, Chairman
R. A. Moore, Co-Chairman
C. J. Bucher
F. B. Queen
F. W. Sunderman

COMMITTEE ON NECROLOGY

H. R. Brown

COMMITTEE ON EDUCATION AND PUBLICITY

W. O. Russell, Chairman
J. L. Carr
W. M. Germain
E. F. Hirsch
D. A. Nickerson
S. P. Reimann

COMMITTEE TO STANDARDIZE EDUCATION AND TRAINING OF MEDICAL TECHNOLOGISTS

J. J. Andujar, Chairman
F. C. Coleman
E. Moss
O. O. Christianson
M. Oosting
R. Miller
P. Rosenblatt
J. P. Tollman

DIFCO

DETECTION OF THE GONOCOCCUS

The superiority of the cultural method over the usual microscopic technique for detection of gonococcal infections has repeatedly been demonstrated. The cultural method is especially indicated in chronic and treated cases and for determining the release of patients. The use of the following standardized materials has simplified the diagnosis of gonorrhoea by the cultural method.

BACTO-G C MEDIUM BASE

enriched with Bacto-Hemoglobin and Bacto-Supplement A or Bacto-Supplement B is the most suitable medium available for cultural detection of *Neisseira gonorrhoeae* from all types of gonococcal infections. This new medium requires only 24 hours incubation. Upon this medium gonococcus colonies may be detected even when the organisms are present in the exudate in such small numbers that they may be missed upon microscopic examination.

BACTO-PROTEOSE NO. 3 AGAR

enriched with Bacto-Hemoglobin is the original medium developed by Difco for isolation of the gonococcus and has been widely used for many years.

BACTO-SUPPLEMENT A
BACTO-SUPPLEMENT B

were developed as enrichments for chocolate agar prepared from Bacto-G C Medium Base or Bacto-Proteose No. 3 Agar with Bacto-Hemoglobin to furnish the essential growth factors required by some strains of *Neisseria gonorrhoeae*.

PROTEOSE PEPTONE NO. 3

is the basic ingredient of Bacto-G C Medium Base and Bacto-Proteose No. 3 Agar. This peptone, in a saline solution, is also recommended as a diluent for suspending the exudate prior to inoculation on chocolate agar.

BACTO-HEMOGLOBIN

in a two per cent solution is the preferred enrichment for preparation of chocolate agar from Bacto-G C Medium Base or Bacto-Proteose No. 3 Agar. It is readily soluble in water and is sterilized in the autoclave.

BACTO-DEXTROSE STARCH AGAR

is a very useful medium for propagation of pure cultures of the gonococcus. The organism grows luxuriantly upon this medium without further enrichment. Bacto-Dextrose Starch Agar also supports excellent growth of many other pathogenic bacteria and when used in half strength it is well suited for maintaining stock cultures.

BACTO-PHENOL RED MEDIA

are particularly useful for determination of fermentative reactions of newly isolated strains of the gonococcus. A selected group of complete agar and broth media are available, as are also the basic media without carbohydrate.

Leaflet No. 121 "Diagnosis of Gonococcal Infections by the Cultural Method" available upon request.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES

DETROIT 1, MICHIGAN

VOLUME 19 SEPTEMBER 1949 NUMBER 9

AMERICAN JOURNAL OF CLINICAL PATHOLOGY

OFFICIAL PUBLICATION OF
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. Anderson
J. J. Andujar
J. E. Ash
L. Berman
G. R. Biskind
O. A. Brines
I. Davidsohn
E. A. Gall
W. M. Germain
A. S. Giordano
G. Gomori
F. W. Hartman
J. W. Kernohan
J. A. Kolmer
H. J. Linn
T. B. Magath
A. R. Moritz
S. P. Reimann
S. B. Rose
A. H. Sanford
E. W. Schultz
F. W. Sunderman
W. S. Thomas
E. Wiebsey

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

Copyright, 1949, by The Williams & Wilkins Company
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Agents: For the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Australia: Angus and Robertson, Limited, 89-95 Castlereagh Street, Sydney; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 SEPTEMBER 1949 NUMBER 9

CONTENTS

Degenerative Changes in the Right Half of the Liver Resulting from Intra-Uterine Anoxia. P. GRUENWALD 801

Adhesiveness of Blood Platelets in Thromboembolism and Hemorrhagic Disorders. II. Diagnostic and Prognostic Significance of Platelet Adhesiveness. S. E. MOOLTEN, L. VROMAN AND G. M. S. VROMAN 814

Case Reports

Neurinoma of the Small Intestine. Its Relative Frequency among Tumors of that Region. P. E. WALKER 827

Bronchial Adenoma with Cancerous Transformation. E. F. GEEVER, W. S. WILLIAMS AND J. E. MCWILLIAMS 836

Paracolon Endocarditis. I. A. FRIEDMAN AND M. GOLDIN 840

Dilatation of the Acini of the Pancreas in Hyperemesis of Pregnancy. Ph. H. HARTZ 846

Book Reviews 850

Selected Abstracts 856

News and Notices 859

Technical Section

Co-Thromboplastin Assay. A Means of Study of Abnormalities of Blood Coagulation. F. D. MANN 861

Recent Advances in the Culture Diagnosis of Fungus Disease. M. L. LITTMAN, A. L. MCQUOWN AND J. D. SCHNEIDAU 865

A Frozen Section Technic. J. B. HAZARD AND G. F. STEVENSON 873

Use of Maxted's Streptomyces Filtrate for Rapidly Extracting Group Carbohydrates from Hemolytic Streptococci. D. LONGFELLOW, S. G. CARY AND L. R. KUHN 880

Use of Köhler Illumination for Reticulocyte Counts. J. D. KENNEDY 883

Nomograph for Rapid Calculation of Icterus Index When Determined by Colorimeter. R. J. HOSLEY 884

Determination of Para-Aminosalicylic Acid Blood Levels. B. J. WILSON AND B. W. ARMSTRONG 886

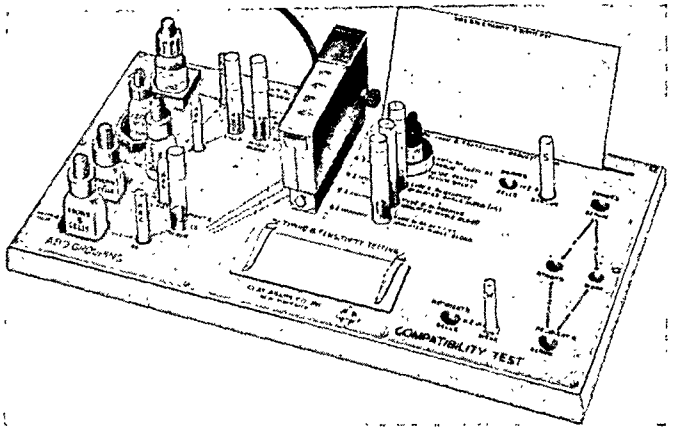
Use of Sponge in Obtaining Tissue from Stomach for Biopsy. S. A. GLADSTONE 891

New Methylene Blue as a Reticulocyte Stain. G. BRECHER 895

An Instrument to Level Tissue in Paraffin Embedding. M. MILLIGAN. 897

Trichrome Stain for Tissue Section, Culture or Smear. M. SANO ... 898

Prevent dangerous errors in blood typing with the BROWN BLOOD BOARD



- **Minimizes the possibility of technical or clerical errors.**
- **Embodies all the latest approved technics of blood grouping, Rh typing, Rh sensitivity testing and cross matching for transfusion.**
- **Simplifies and combines these technics in an orderly sequence.**
- **Facilitates the grouping and cross matching of blood for emergency or routine use.**
- **Can be used for routine Rh typing and sensitivity testing in individual maternity cases.**

The Brown Board[1] was developed in the Duke University Hospital Blood Bank to increase efficiency and reduce errors in blood typing and cross matching. It consists of an etched and stamped metal plate on a hardwood base, with an Rh typing box, anti-serum bottle adapters, reagent bottles, test tubes, dropping pipettes and concavity slides for Rh typing.

Tubes and reagent bottles have in-the-glass labels in different colors, matching the labelling and coloring of their places in the Board. Each reagent bottle and cross matching tube is of a different shape or size, so that it fits only in its proper place. Antiserum bottle adapters hold any commercial anti-serum bottle; they are permanently marked, fit only in their own place in the Board and are colored to conform to the coloring recommended for the sera by the National Institute of Health. Reagent bottles and adapters are designed to permit one-hand removal and replacement of the dropping pipettes.

The Rh typing box[2] is lighted, tiltable and designed to maintain a temperature of 37° to 42° C in the slide concavity where the blood sample is placed.

The Board has a slot to hold the Donor Card and Transfusion Request Form directly in front of the technician while tests are being made. A blotting paper holder is also provided, conveniently near the technician.

Refs: 1. "A Note on Blood Grouping and Cross Matching with Special Reference to a Convenient Cross Matching Board." Brown, I. W., Jr., M.D. In press.

2. "The Demonstration of Anti-Rh Agglutinins—An Accurate and Rapid Slide Test," Louis K. Diamond, M.D., Boston, Mass., and Neva M. Abelson, M.D., Philadelphia, Pa., Journal of Laboratory and Clinical Medicine, Vol. 30, No. 3, March 1945.

A-2800 Brown Blood Board, complete with Rh typing box, anti-serum bottle adapters, reagent bottles, test tubes, dropping pipettes and four-concavity slides. **Each $90.00**

A-2801 Brown Blood Board, same as above but with basic equipment only. **Each $56.00**

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

(In writing to advertisers, please mention the Journal—it helps.)

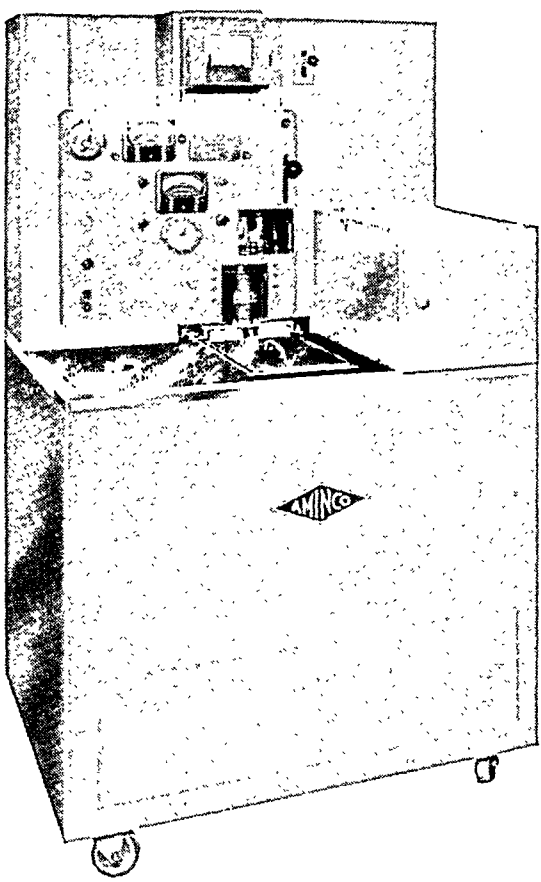
Far ahead of the field!

AMINCO TERN

it will pay you to investigate the MOST ADVANCED AND COMPLETE instrument for Electrophoretic analysis

ELECTROPHORESIS APPARATUS

Exclusive Features
NOT TO BE FOUND ELSEWHERE



- Most economical because of greater analytical capacity.
- Most useful for research and routine work.
- High sensitivity . . . can detect protein concentrations as low as 0.002%.
- Operates continuously regardless of atmospheric conditions, except for servicing.
- Most complete . . . includes rapid dialysis facilities, chamber for freezing and storing specimens, sealed mechanical refrigeration system, precision Schlieren optics, complete photographic facilities, and a rotatable triple diaphragm for selecting slit, bar and straightedge patterns.
- Compact . . . measures only 30 x 37 x 67 inches high. Moved about with ease.
- More convenient . . . operator works in front of the instrument. All parts at convenient heights and locations.
- Shipped ready for operation.
- Accommodates semi-micro, macro, macropreparative, adsorption, and conductivity cells.
- Engineered for long life. Components rated far beyond their normal operating requirements.

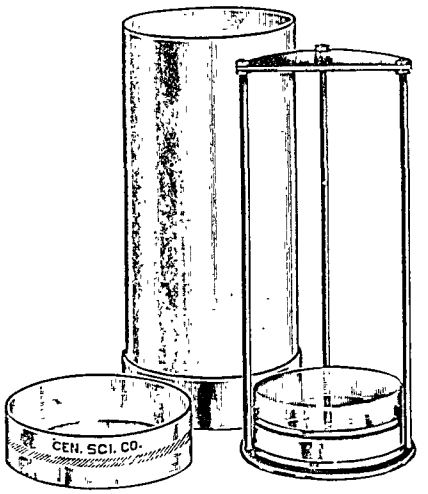
BULLETIN 2175-P
TELLS THE ENTIRE STORY
Write for your copy

Since 1919 AMINCO

AMERICAN INSTRUMENT CO., Inc.
Scientific Instruments
SILVER SPRING, MARYLAND
IN METROPOLITAN WASHINGTON, D. C.

CENCO

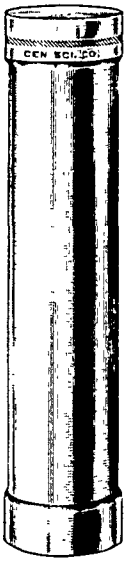
PETRI DISH HOLDER



Cenco Cylindrical Culture Dish Holder will hold 100 mm. round Petri dishes during sterilization or incubation. Its capacity is 16 dishes 100 ×10 mm. or 12 dishes 100 × 15 mm. Consists of a cylindrical box, made of seamless aluminum tubing with machined cast-aluminum bottom and telescoping cover, and a removable frame with aluminum end pieces for convenient carrying of the Petri dishes. Dimensions: Length, 10⅜ inches; diameter, 4⅞ inches.

No. 44398. Culture Dish Holder, Cylindrical Form.....Each **$7.25**

PIPETTE BOX



Cenco Cylindrical Pipette Box is designed for holding bacteriological pipettes during sterilization, or for storage. Made of heavy, seamless aluminum tubing with machined cast-aluminum bottom and telescoping cover. Dimensions, inside: Length, 16 inches; diameter, 3 inches.

No. 46665. Pipette Box, Cylindrical.....Each **$5.15**

SERVING SCIENCE CENCO

CENTRAL SCIENTIFIC COMPANY

Scientific Apparatus • Instruments • Chemicals

1700 IRVING PARK ROAD, CHICAGO 13

NEW YORK BOSTON SAN FRANCISCO NEWARK LOS ANGELES TORONTO MONTREAL

(In writing to advertisers, please mention the Journal—it helps.)

Ortho anti RH Sera

These are the factors which govern the efficiency and usefulness of Anti-Rh Sera. Ortho Anti-Rh Sera, because they are prepared by skilled technicians from carefully selected human bloods are accurate, potent, avid, and stable.

Available:

1. Anti-Rh_0 (Anti-D) Typing Serum for the Slide Test
 5 cc. vials (Approximately 200 tests)
2. Anti-Rh_0 (Anti-D) Typing Serum for the Test Tube Test
 2 cc. vials (Approximately 75 tests)
3. Anti-Rh_0" (Anti-CD) 87% Typing Serum for the Test Tube Test
 2 cc. vials
4. Anti-rh' (Anti-C) Typing Serum for the Test Tube Test
 2 cc. vials
5. Anti-rh" (Anti-E) Typing Serum for the Test Tube Test
 2 cc. vials
6. Anti-Human Serum for the Coombs "Developing" Test
 2 cc. vials

Anti-Human Serum for Coombs "Developing" Test

Anti-Human Serum detects the presence of even minute amounts of blocking antibodies, thereby offering a valuable method of detecting immunization due to a wide variety of antigens including the Rh factor.

Anti-Human Serum is useful in:

1. Detecting maternal Rh antibodies in cord blood of infants.
2. Checking the bloods of patients suspected of being immunized against the Rh or other factors.
3. Detection of those antibodies which fail to agglutinate saline suspended donor cells as in cross-matching for transfusion.
4. Study and diagnosis of certain acquired hemolytic anemias.

Packaging:
2 cc. vials with a standardized dropper.

Ortho

ORTHO PHARMACEUTICAL CORP., RARITAN, N. J.

Bausch & Lomb Simplified Phase Contrast Accessories

with { 1. Long Working Distance Condenser
2. Turret-type Abbe Condenser

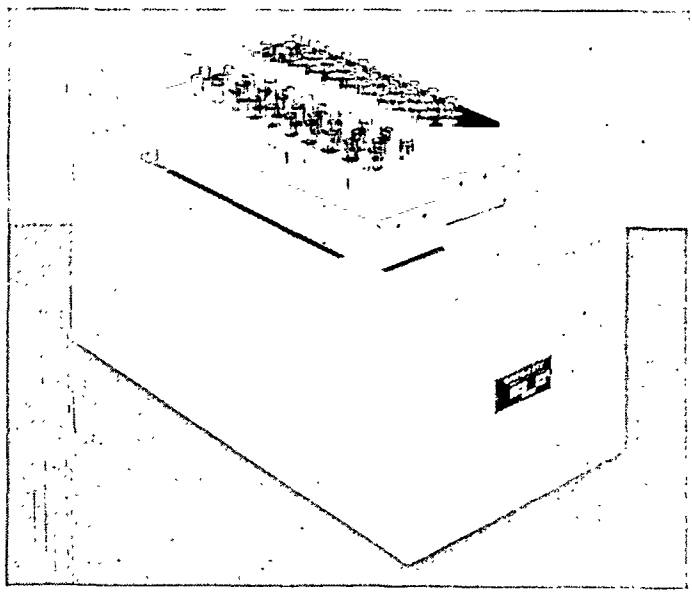


You requested it and here it is! The Individual Annular Stop *Long Working Distance* Condenser . . . for phase contrast examination of tissue cultures in flasks or chambers. Provides a long working distance . . . from 8mm to 10mm in air. Designed for quick, easy mounting on either Bausch & Lomb Research or Laboratory Microscopes. Utilizes *simplified* accessories similar to the turret-type. You get the same *top image contrast* from ¼ wavelength annulus, positive contrast image, and balanced intensity of direct and refracted light beams . . . and the same speed, simplicity, and ease of operation.

New!

WRITE for your copy of Catalog D-104, just off the press. It contains important, newly published information on phase contrast principle and practice, as well as new complete information on both types of Bausch & Lomb Phase Contrast Accessories. Bausch & Lomb Optical Company, 652-I St. Paul Street, Rochester 2, New York

BAUSCH & LOMB *Phase Contrast* ACCESSORIES



For large installations two or more of these instruments may be banked together.

Improved SHAKING APPARATUS

FOR THE KAHN TEST

• QUIET OPERATION • MINIMUM VIBRATION

An improved model of the original Sargent Spring Suspended Shaking Machine, with vibration further reduced by the introduction of reciprocating movement of the carriages.

The carriage in which Kahn tube racks are held is supported by tempered steel springs which by slight flexure allow the carriages to be moved through their longitudinal axes by the specified amount of 1½ inches. This device replaces former systems of moving frames operating as articulated parallelograms and sliding carriage systems, the difference in degree of silence and momentum of oscillation being very marked.

Power requirement is much lower than in older forms and the carriers are driven by a small induction type electric motor, silent and smooth running.

The entire base, cover and carriages of this unit are finished in permanent baked white enamel. Other exposed metal parts are chromium plated. The apparatus is thus highly sanitary and easy to keep clean.

No. 7935 rubber plated Kahn tube racks are particularly recommended for use with this machine and are held firmly in the carriers by end pressure springs.

Small and light, this unit is easily handled by technicians. For this reason and because of its quiet operation and freedom from vibration, it is recommended for use in hospital laboratories in preference to heavier, more noisy units. Length, 18 inches; height, 10 inches; width, 9 inches; shipping weight, 40 pounds.

S-7880 SHAKING MACHINE—Kahn, Sargent. Without tube racks, with rubber covered cord and plug for attachment to standard outlet for operation from 115 volt, 50/60 cycle, circuits..... $80.00

SARGENT

SCIENTIFIC LABORATORY EQUIPMENT AND CHEMICALS

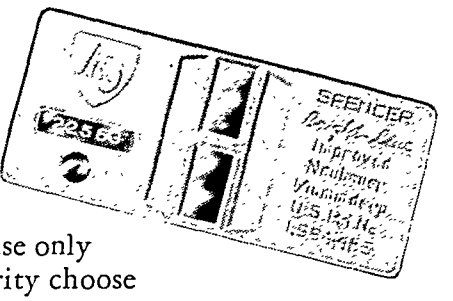
E. H. SARGENT & COMPANY, 155-165 EAST SUPERIOR ST., CHICAGO 11, ILL.

MICHIGAN DIVISION 1959 EAST JEFFERSON DETROIT 7, MICHIGAN

SOUTHWESTERN DIVISION 5915 PEELER ST. DALLAS 9, TEXAS

(In writing to advertisers, please mention the Journal—it helps.)

Only an ACCURATE Blood Count is Worth Taking



To aid in correct diagnosis and proper treatment of patients, a blood count *must* be accurate. That is why most technicians follow accepted techniques with utmost care . . . why they use only the finest equipment . . . why the majority choose the famous Spencer "Bright-Line" Haemacytometer.

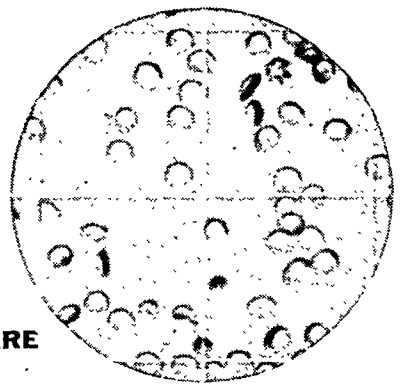
Only BRIGHT-LINE Chambers offer these 6 Safeguards to Accuracy

GREATER CONTRAST BETWEEN CELLS AND BACKGROUND

Cells appear dark, lines bright, background gray. Each cell is easily counted—even those which overlap lines.

MORE EVEN DISTRIBUTION OF CELLS

Surface tension differences between metalized surface of chamber and glass surface of cover cause cells to distribute more evenly and chamber to fill more readily.



SIMULTANEOUS RESOLUTION OF BLOOD CELLS AND RULINGS

It is not necessary to stop down the condenser of your microscope since blood cells and rulings can be clearly resolved at full diaphragm opening.

METALIZED BACKGROUND ELIMINATES GLARE

Absence of glare provides clearer vision and reduces eyestrain.

INDENTATION OF UNDERSIDE PROLONGS USEFUL LIFE

Introduction of a slight concavity directly under rulings prevents scratches appearing on lower surface of chamber. Such scratches impair counting accuracy.

PRODUCTION TOLERANCES ARE HELD TO THOSE RECOMMENDED AND SET FORTH BY THE U. S. BUREAU OF STANDARDS

American Optical
COMPANY
Scientific Instrument Division
Buffalo 15, New York

When you buy, specify "Bright-Line" and accept no substitutes. Immediate delivery from your supplier or write Dept. W-16.

Makers of Medical Optical Instruments for Over 100 Years

SPENCER



pipette washing can be as

easy as this

No repeated handling of pipettes when you're using this efficient TECHNICON washer . . . they're gathered, cleansed, washed, and dried *in the same basket.* In three brief minutes, the TECHNICON Pipette Washer delivers a *sparkling-clean* basketload (200 at a time, 1 ml in 100th's . . . and it'll take them up to 18" long).

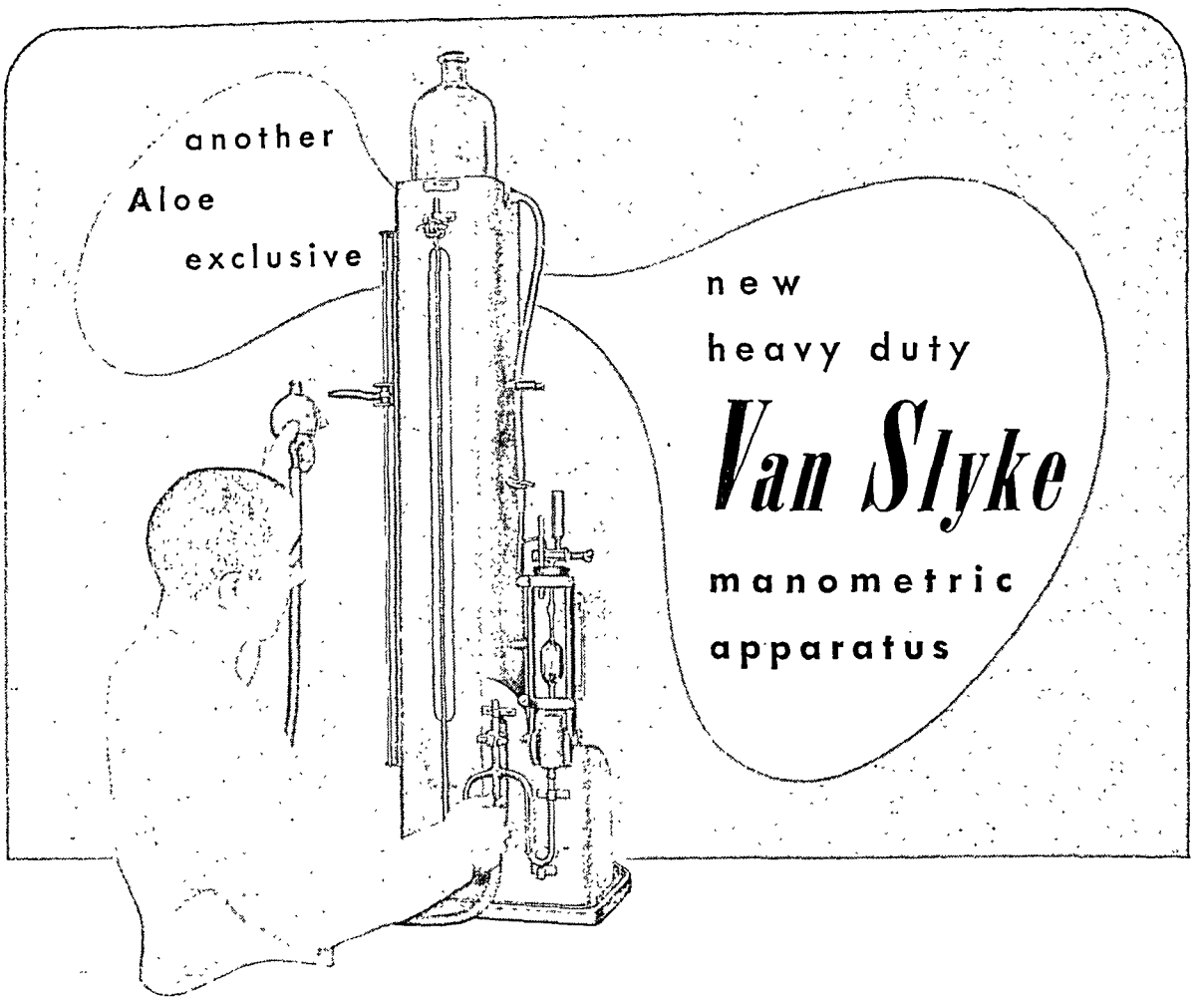
echnicon

automatic PIPETTE WASHER

We will be glad to send Bulletin #6150 giving particulars.

THE TECHNICON COMPANY

215 EAST 149th ST. • NEW YORK 51, N. Y.



Modern, All-Metal, Fully Enclosed, More Convenient

This streamlined manometric apparatus contains new features for more accurate analysis and is equipped with Van Slyke-Folch extraction chamber for use in carbon determinations as well as gas analysis.

The entire shaking mechanism is enclosed in an all-metal, seamless housing. Motor, wiring, and driving gears are protected against spillage and fumes. The perfectly balanced housing and weighted base insure quiet operation. Crevices are minimized to prevent trapping of mercury.

Speed of ball-bearing motor, controlled by an improved rheostat, permits exact adjustment at any desired point. Additional refinements for the operator's convenience include a trough for recovering mercury . . . individual fluorescent lights for reading manometer and extraction chamber . . . a rack-type slide holder for raising and lowering leveling tube . . . clearly etched graduations . . . gas tight stopcocks, selected for taper and bore, which reduce wear . . . entire apparatus can be carried by one man.

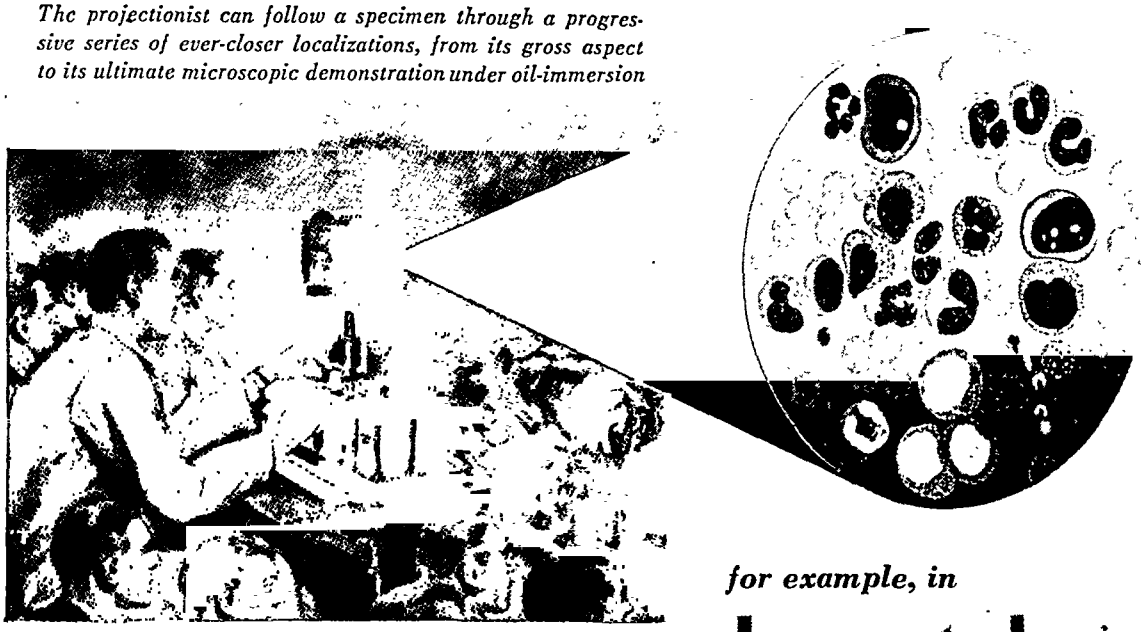
JL13590 Improved Van Slyke Manometric Apparatus, as described with manometer tube, extraction chamber, leveling bulb, aspirator bottle, thermometer, cord and plug but without mercury. For operation on 115 volts, A. C. each. **$285.00**

A. S. aloe COMPANY *Serving Science Since 1860*

1831 Olive Street • St. Louis 3, Missouri

mass demonstration in the auditorium

The projectionist can follow a specimen through a progressive series of ever-closer localizations, from its gross aspect to its ultimate microscopic demonstration under oil-immersion

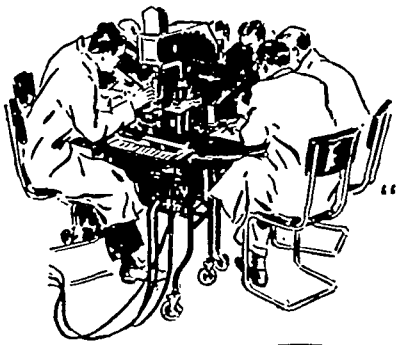


for example, in

hematology

The auxiliary Scopicon dark-chamber "round-table" affords superb facility for small-group study. Here the projected image is cast upon a platen within a light-tight chamber, with separate light-excluding viewing hoods for each observer. Any number up to ten may share in conference, which can be conducted in a normally lighted room. A pointer at each port permits any observer to indicate areas of interest to all the others for discussion.

The 64,000 lumen intensity of the Scopicon high-pressure mercury lamp permits auditorium demonstration of blood smears even under oil-immersed microscope objectives. In projected images six feet or more across, the minute granular details of white blood cells can be seen, due to the pinpoint character of the 1 mm. square (approx.) focal spot employed. The Scopicon light is steady, flickerless . . . its brilliant white color exhibiting biological stains to the greatest advantage. May we send you the brochure describing this versatile instrument?



. . . and for small-group "round-tables" in a normally lighted room

the Scopicon

SCOPICON, Inc.
215 East 149th St., New York 51, N. Y.

microprojection
photomicrography



routine for discovery

The national total of undiagnosed or "unknown" diabetics may run from a million to two or even three.[1,2] Modern treatment, when promptly initiated, can do much to prevent metabolic decompensation and to minimize diabetic complications. Therefore, the clinical revealment of diabetes mellitus at an early stage is essential.

Thus, "all patients who present themselves to the physician for an examination should have a routine urine examination."[3] In this phase of practice, the advantages of *Clinitest*® tablets for urine-sugar analysis are considerable.

Clinitest is dependably accurate, yet it takes only a few seconds to perform. The test is simple—no external heat need be applied; interpretation is by direct color comparison. *Clinitest* is convenient both for the doctor's office routine and for the diabetic patient's prescribed sugar-level checkups.

(1) Joslin, E. P.: Postgraduate Med. 4:302 (Oct.) 1948. (2) Kemper, C. F.: Rocky Mountain M. J. 45:1092 (Dec.) 1948. (3) Pollack, H.: New York Med. 4·15 (Dec. 5) 1948.

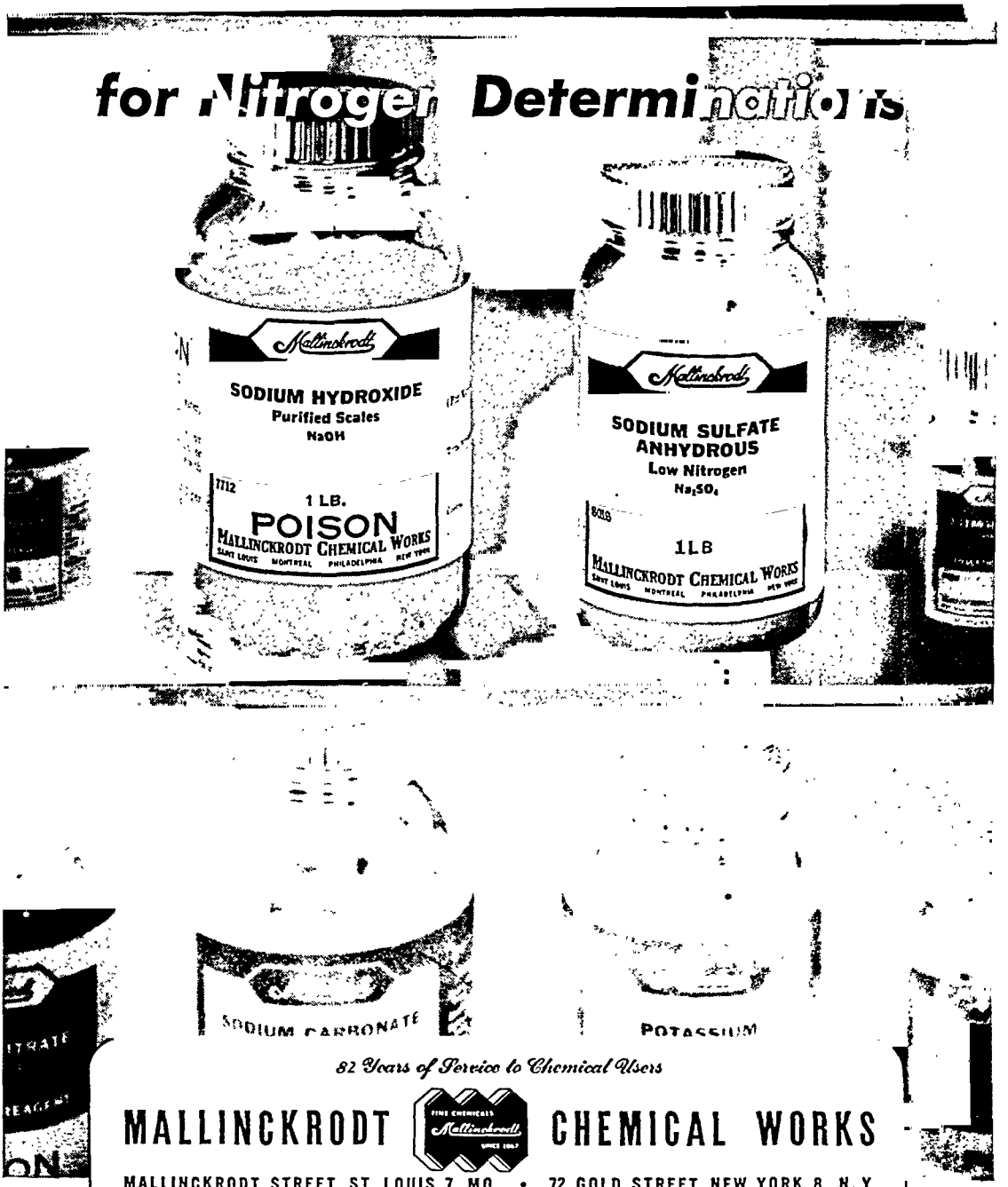
Clinitest

for urine-sugar analysis

AMES

AMES COMPANY, INC. • ELKHART, INDIANA

(In writing to advertisers, please mention the Journal—it helps.)



82 Years of Service to Chemical Users

MALLINCKRODT CHEMICAL WORKS

MALLINCKRODT STREET, ST. LOUIS 7, MO • 72 GOLD STREET, NEW YORK 8, N.Y.

CHICAGO • CINCINNATI • CLEVELAND • LOS ANGELES • MONTREAL • PHILADELPHIA • SAN FRANCISCO

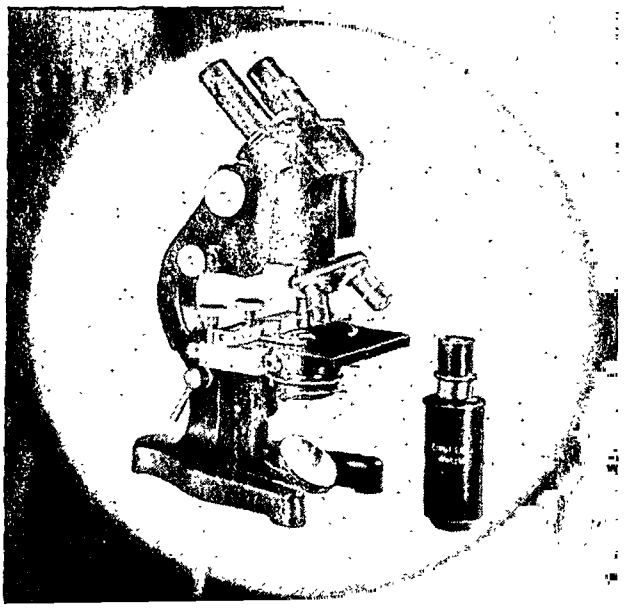
UNIFORM DEPENDABLE PURITY

(In writing to advertisers, please mention the Journal—it helps.)

Available now...

Leitz

Medical Microscopes



Leitz

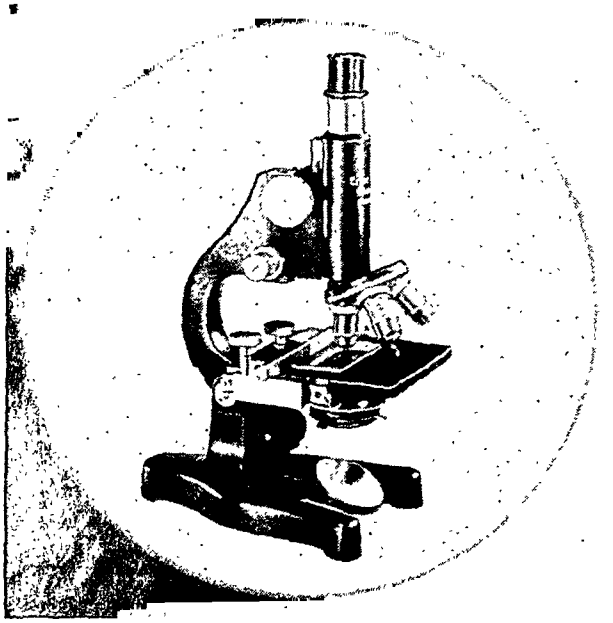
BINOCULAR MICROSCOPE BST 25/92

(Interchangeable monocular tube with draw tube available)

The ideal instrument for the medical profession offering:

Ball-bearing fine adjustment
Built-on, ungraduated mechanical stage
Quadruple nosepiece
3 Achromatic Objectives: 16 mm., 4 mm., 1/12th oil
2 pairs of eyepieces
Magnifications 60-1000X

$476



Leitz

MONOCULAR MICROSCOPE CT 25/92

The preferred student and laboratory microscope featuring:

Sturdy construction
Unsurpassed optical performance
Smooth-operating fine adjustment without gears
Built-on, ungraduated mechanical stage
Magnifications 60-1000X

$298

Write today for Catalog Micro—100 B

E. LEITZ, Inc., 304 Hudson St., New York 13, N. Y.

LEITZ MICROSCOPES • BINOCULARS • SCIENTIFIC INSTRUMENTS
LEICA CAMERAS AND ACCESSORIES

(In writing to advertisers, please mention the Journal—it helps.)

FIVE

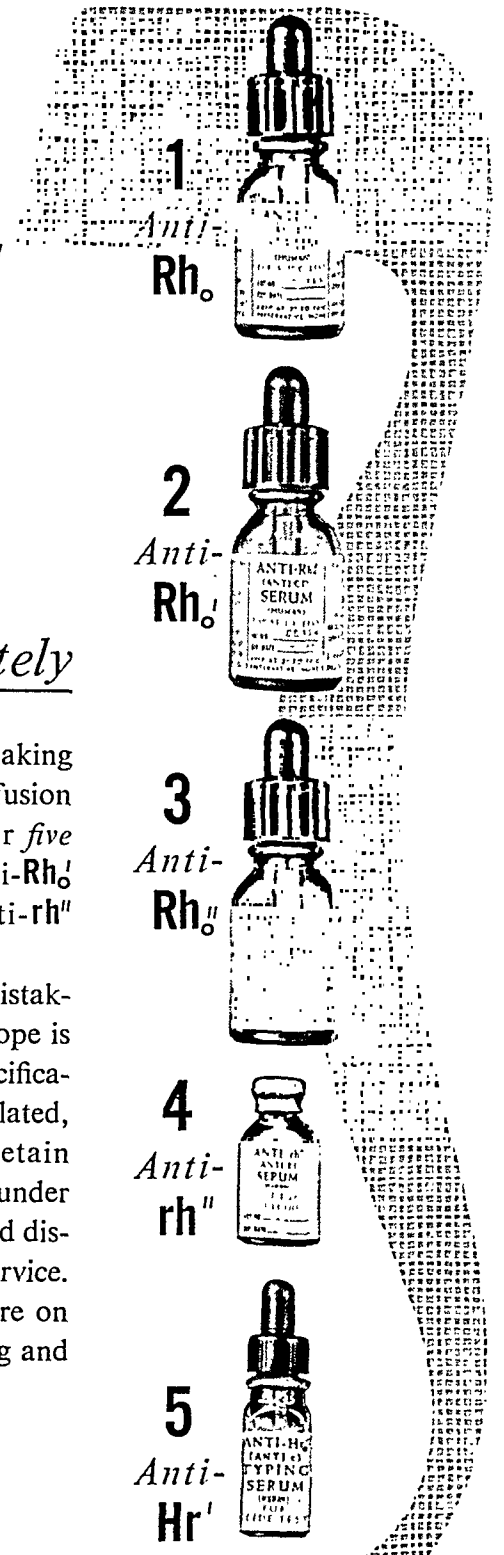
Anti-(Rh) Serums

to test every blood—completely

More and more pathologists are making *complete* **Rh** determinations for blood infusion cases. They're turning to American for *five* potent serums—Anti-Rh_o (Anti-D), Anti-Rh_o' (Anti-CD), Anti-Rh_o'' (Anti-DE), Anti-rh'' (Anti-E) and Anti-Hr' (Anti-c).

The slide method gives accurate, unmistakable readings in two minutes. No microscope is needed. Serums, prepared to N. I. H. specifications, may be used with fresh, clotted, oxalated, heparinized or citrated blood. They retain original potency over a long period even under adverse conditions. Six strategically located distributing centers assure you of prompt service.

WRITE today for descriptive literature on AMERICAN'S complete line of blood-typing and blood-grouping serums.





PLAN WITH AMERICAN
... the first name in hospital supplies

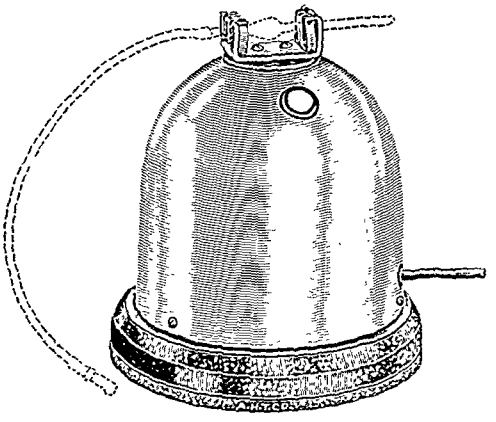
AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

A.H.T. CO. SPECIFICATION

BLOOD PIPETTE SHAKER

With automatic shaking control and shutoff, providing quick, effective and reproducible shaking



3426-A.

SHAKER FOR HEMACYTOMETER PIPETTES, Research Model, Electric, A.H.T. Co. Specification. For quick and effective shaking of diluting pipettes in a reproducible manner. The automatic shaking action, requiring approximately thirty seconds, comprises 1200 rapid, transverse vibrations of small amplitude, with automatic shutoff, reducing the possibility of hemolysis due to mechanical rupture and fragmentation of fragile erythrocytes.

Consisting of a shaded-pole electric motor fitted with eccentric drive and spring device for converting continuous into vibratory motion; gear train with cam and micro-switch for limiting the predetermined number of vibrations; all enclosed in dome-shaped aluminum housing on cast metal base, with push button for starting, and aluminum pipette holder with nickel-plated spring bronze clips.

The pipette holder is a simple device which permits insertion or removal of pipette with one motion. The type and amplitude of the stroke make it possible to shake the pipette efficiently with both ends open but, if preferred, shaking can be accomplished without removing rubber tubing, mouthpiece or closure. Overall dimensions 6½ inches diameter x 8 inches high; current consumption 0.1 ampere.

CAUTION. Blood cells begin to settle immediately after vibration ceases and delay in filling the counting chamber, particularly for red cell count, will introduce appreciable error. While this Shaker is offered with a two-place head, *we do not recommend shaking more than one pipette at a time* except when making routine counts of red and white cells on the same specimen, or when used simultaneously by more than one technician.

3426-A. Shaker for Hemacytometer Pipettes, Automatic, Research Model, Electric, A.H.T. Co. Specification, as above described, with two-place head, thick sponge rubber pad, 5-ft. connecting cord, switch and plug. For use on 115 volts, 60 cycles, single phase, a.c. only..... 40.00
10% discount in lots of 6 or more.

ARTHUR H. THOMAS COMPANY
RETAIL—WHOLESALE—EXPORT
LABORATORY APPARATUS AND REAGENTS
WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.
CABLE ADDRESS, "BALANCE", PHILADELPHIA

DEGENERATIVE CHANGES IN THE RIGHT HALF OF THE LIVER RESULTING FROM INTRA-UTERINE ANOXIA*

PETER GRUENWALD, M.D.

From the Departments of Pathology, Long Island College of Medicine and Kings County Hospital, Brooklyn, New York

In the course of autopsies on newborn infants a difference in the appearance of the two lateral portions of the liver was found in several instances. There was a sharp dividing line which took a zig-zag course, roughly parallel to the falciform ligament, and 1 to 2 cm. to the right of it. A similar dividing line was seen on the cut surface. The two portions of the liver were approximately of equal size, since a greater part of the liver is normally present to the right of the falciform ligament. The difference noted grossly between the halves of the liver was confirmed by a difference in the histologic appearance, as will be described below. In subsequent autopsies attention was centered on similar findings, and inconspicuous differences between halves of the liver were then found which previously would not have been noted. A standard method was used in obtaining tissue from the liver for microscopic examination so that sections would contain areas on both sides of the dividing line. A block was cut from a transverse slice, extending from the falciform ligament to the right for about 3 cm., and the right side was marked by a notch which then appeared in the finished sections. This method was also used in livers which grossly showed no dividing line, and additional instances were thus discovered on microscopic examination.

Pertinent changes noted in cases with a difference between the halves of the liver are compiled in Table 1. It is impossible to give the exact incidence of the finding under consideration, since the attention given to this point varied throughout the period of collection of this material. The following data will give an approximate indication of the incidence. They are based on autopsies on stillborn infants and infants aged 2 months or less. During a period in which grossly visible differences within the liver were sought for, approximately 200 such autopsies were performed, and a dividing line was found in 20 instances and confirmed microscopically. In about 90 instances in which no such line was seen, liver sections were prepared as described above, and microscopic examination revealed 10 additional cases with a difference in the appearance of the two sides not seen grossly. The incidence is thus at least 15 per cent.

Microscopically, various degenerative changes were seen either only in the right half of the liver or more severely in the right than in the left half. The changes, as listed in Table 1, included cloudy swelling (Figs. 1, 2) or fat-free vacuolation (Fig. 9) of the cytoplasm, fatty metamorphosis (Figs. 1, 4, 5, 9),

* Publication No. 6 of the Brooklyn Survey of Neonatal Pathology. A number of hospitals in Brooklyn contributed pathologic material for this survey, which is supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

Received for publication, May 13, 1949.

TABLE 1

DATA ON 44 INFANTS WITH LIVER DAMAGE PREDOMINANT IN THE RIGHT HALF OF THE ORGAN

INFANTS			DIFFERENCE IN LATERAL HALVES OF LIVER (RIGHT/LEFT)*							SIGNS OF ASPHYXIA			
Case No.	Age	Length in Cm.	Grossly Visible	Cloudy Swelling	Fat-free Vacuoles	Fatty Change	Atrophy	Focal Necrosis	Iron Deposit	Aspirated Vernix	Petech. Hemorrhage	Necrosis in Artery	Esophagitis, Gastric Erosion
Stillborn:													
1	stillborn	34.5	+	+/+	−/−	−/−	+++/−	−/−	−/−	−		−	−
2	"	37	+	+/+	++/−	−/−	−/−	−/−	−/−	−	+	−	−
3	"	38	+	−/−	+/+	−/−	++/t	−/−	−/−	−	+	−	−
4	"	38	+	+/+	++/+	+++/+	−/−	−/−	−/−	+	+	−	
5	"	42	+	+/−	+/t	+/t	−/−	−/−	−/−	−	+	−	−
6	"	43	+	+/+	−/−		−/−	−/−	++/t	−		−	
7	"	45.5	+			+/t			−/−	+			
8	"	48	+	+/+	+/t	+/−	++/−	+/−	−/−	+	+	+	+
9	"	48	+	−/−	−/−	++/−	−/−	+/+	−/−	+	+	−	
10	"	49	+				+/−		−/−	+	+	−	
11	"	49	+	t/t	++/t	+/−	−/−	−/−	−/−	+	+	+	
12	"	50.5	+	−/−	−/−	+++/+	−/−	−/−			+		
13	"	51	+	+/−	+/+	++/t	−/−	−/−	−/−	+	+	−	−
14	"	51	+			+++/+			−/−	+		−	
15	"	51.5	+	++/t	−/−	−/−	−/−	−/−	t/t	+	+	−	+
16	"	52	+	+/+	−/−	++/t	−/−	−/−		+	+	−	
17	"	53.5	−	−/−	−/−	+/−	−/−	−/−	−/−			−	−
18	"	54.5	+			++/t			t/t	+	+	−	−
19	"	56	+	++/t	+/t	+/t	−/−	−/−	+/t	+	+	−	+
20	"	57	−	+/−	−/−	++/t	−/−	−/−	−/−	+	+	−	−
Liveborn, less than 10 days old:													
21	7 hours	48.5	−	+/t	++/++	−/−	−/−	−/−	−/−	−	+	−	−
22	9 "	49.5	+	+/+	−/−	−/−	−/−	−/−	++/+	+	−	−	−
23	16 "	51	−	+/+	t/−	t/−	−/−	−/−	−/−	−	+	+	+
24	19 "	51	+	t/t	++/−	++/−	−/−	−/−	−/−	+	+	+	+

25	21 "	51	+	−/−	+/−	+/−	−/−	++/−	−/−	+	+	+	+
26	24 "	52	+	++/t	+/+	−/−	−/−	−/−	−/−	+	+	−	−
27	24 "	54	+	+/−	+/t	++/t	−/−	−/−	−/−	−	+	−	+
28	24 "	55	+	++/−	−/−	+/t	−/−	−/−	−/−	+	+	+	+
29	30 "	44	−	++/t	−/−	+/−	−/−	−/−	−/−	−	−	+	+
30	31 "	51	+	+/+	+/+	+++/+	−/−	++/−	−/−	+	+	−	+
31	32 "	42	+	?/−	?/−		+++/−	+++/−		−	−	−	
32	34 "	37	−	−/−	+/t	−/−	−/−	++/−	−/−	−	+	+	−
33	2 days	44	+	++/+	−/−		−/−	++/+	+/+	−	+	−	+
34	2 "	48	−	+/t	t/t	−/−	−/−	−/−	+++/+	+	+	+	+
35	2 "	48	+	t/t	+++/t		−/−	−/−	++/t	−	+	+	+
36	2½ "	41	−	+/+	++/t	t/t	−/−	−/−	−/−	−	+	−	+
37	3 "	47	−	+/+	−/−	++/t	−/−	−/−	t/t	+	+	−	+
38	3 "	50.5	−	−/−	+/+	+++/++	−/−	−/−		−	−	−	
39	4 "	49	−	++/+	−/−	+++/+	−/−	−/−	−/−	−	−	−	+
40	8 "	47	+	−/−	+/t	++/t	−/−	−/−	+/+	−	+	−	−
Liveborn, 1 to 2 months old:													
41	4 weeks	47	+	−/−	−/−	++/+	−/−	−/−		−	−	−	−
42	7 "	47	−	−/−	−/−	++/++	−/−	−/−	++/t	−	−	−	−
43	7 "	51	+	+/+	++/++	++/+	−/−	++/−	−/−	−	−	−	−
44	8 "	57	+	+/+	+/+	++/+	−/−	−/−	+/t	−	−	−	−

* The estimated intensity of changes is given as ranging from + to +++. Negative findings are entered as −; t refers to "trace", or a very mild change. When no observation was recorded, the respective space is left open.

hemosiderosis (Fig. 6), atrophy with severe congestion (Fig. 7), or multiple foci or necrosis (Figs. 3, 12). Cloudy swelling appeared either in the form of granular cytoplasm (Fig. 1), or as a very pale cytoplasm with a few granules and a distinct cell border (Fig. 2). The distinction of fatty and fat-free vacuoles was made in the majority of cases by examination of frozen sections stained with Sudan III; in some others in which material for fat stain was no longer obtainable, it was felt that routine paraffin sections permitted the identification. The presence of iron-containing pigment was recognized only in the presence of a positive Prussian blue reaction. Atrophy manifested itself by very thin liver cell plates separated from one another by wide sinusoids (Fig. 7). At the first glance there was the appearance of severe, chronic venous congestion. However, inasmuch as the left half of the liver did not show this severe change, and since there was no known cause or other indication of extreme congestion, it appears that the condition is largely one of atrophy of liver cells. A similar explanation has been given for the shrinkage of cells in chronic congestion of the liver.[1]

The findings in a few representative cases will be described. Case 3 was that of a stillborn male infant measuring 38 cm., and weighing 1300 grams. The mother bled for two weeks prior to spontaneous delivery. A tentative diagnosis of premature separation of the placenta was made. The last fetal movements were felt two days before delivery. The infant was only slightly macerated and showed moderate edema and general venous congestion. Focal hemorrhages were present in the epicardium, pleura and testes; the heart was dilated; the lungs showed partial expansion of the alveoli with fluid. On the surface of the liver a faint difference in appearance was seen between the right and left halves, with a borderline somewhat to the right of the falciform ligament. On section this difference was more conspicuous; the left half was firmer and paler red than the right. Microscopically the liver cells in the left half of the organ were of average size and contained small vacuoles in the cytoplasm. In the right half the cell cords were very thin, showing atrophy as mentioned above (Fig. 7).

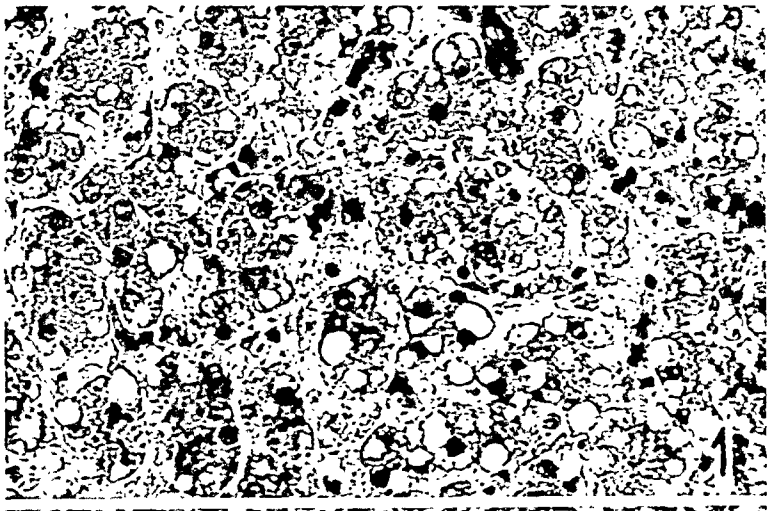
In Case 10 the infant was somewhat more macerated. It was a male, measured 49 cm. and weighed 2600 grams. Autopsy revealed hemorrhages in the epicardium, pleura, thymus and brain. The lungs contained aspirated vernix caseosa, and were the seat of pneumonia. The liver showed externally a striking difference between its halves; the left half was paler and firmer than the right (Fig. 8). Microscopic examination was unsatisfactory owing to severe postmortem changes.

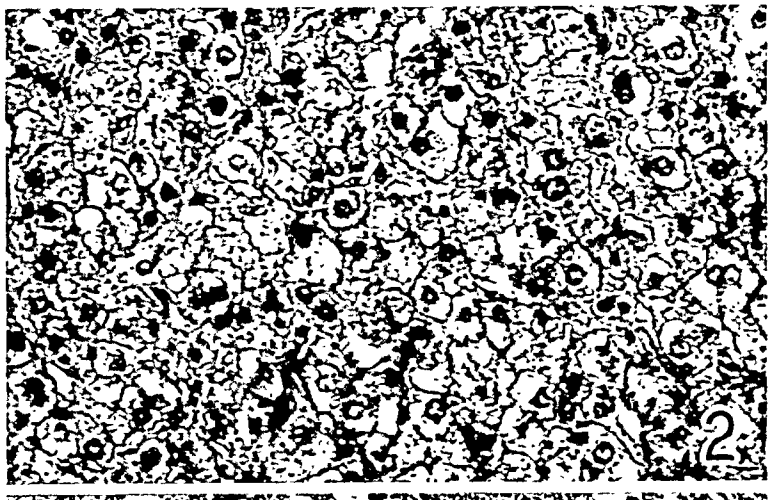
Case 24 was that of an asphyxiated infant born after long labor and surviving for a short time. The mother was in weak labor for 48 hours, and was delivered by cesarean section because of cephalopelvic disproportion. The infant did poorly, and upon arrival in the nursery gave the impression of being lifeless. With the use of various methods of resuscitation it was kept alive for 19 hours, but it never cried properly. At autopsy the body measured 57 cm. and weighed 3260 grams. There was general congestion, and dilatation of the heart. All the conditions believed to be indicative of asphyxia, which are listed in Table 1, were present, as well as pneumonia and focal necrosis in the adrenals. The liver showed on the surface and on section a sharp line of demarcation of its halves; the left half was somewhat darker red and firmer than the right. There was moderate cloudy swelling of the liver cells. In the right half of the organ the cytoplasm contained numerous vacuoles of various

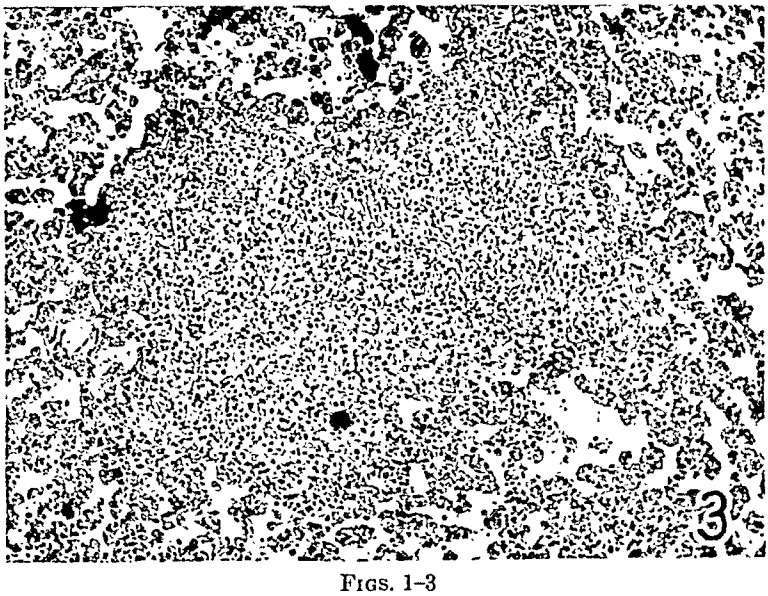
FIG. 1. Case 39. Section from the right half of liver, showing granular cytoplasm of liver cells with large fat vacuoles. × 400.

FIG. 2. Case 15. Section from right half of liver. Liver cells have very pale cytoplasm and distinct cell borders. × 400.

FIG. 3. Case 32. An area of necrosis in right half of liver. × 125.







FIGS. 1–3

sizes (Fig. 9); some of these contained fat as verified in frozen sections. No vacuoles were present in the cells of the left half (Fig. 10).

Case 25 was that of an infant whose liver showed the most striking gross changes in the right half; they are inadequately reproduced in Figure 11. This male infant was born spontaneously after 12 hours of labor. It breathed irregularly and was intermittently cyanotic. The body was flaccid except for right-sided convulsions. The infant expired one day after birth. At autopsy the body measured 51 cm. and weighed 3100 grams. As in the previous case, it showed general congestion, dilatation of the heart, aspiration of vernix caseosa, pneumonia, and the pathologic changes listed in Table 1; esophagitis was moderate. On the surface of the liver there was a line of demarcation 2 cm. to the right of the falciform ligament. The right half was yellowish in color, and the left half pale red. On section, the right half showed within the yellow tissue scattered hemorrhagic areas (Fig. 11). Frozen sections revealed a moderate amount of fat only in the right half of the organ. Paraffin sections showed scattered areas of necrosis having no typical relation to the portal fields or central veins (Fig. 12). There was extensive necrosis in the wall of many arteries throughout both halves of the liver.

Case 31 was described in detail elsewhere[3] with regard to rupture of the liver and hemoperitoneum. The right half of the liver showed histologically complete hemorrhagic necrosis. Rupture occurred over this area on the second day of life. This case possibly represents a severe or later stage of atrophy than that found in Case 3.

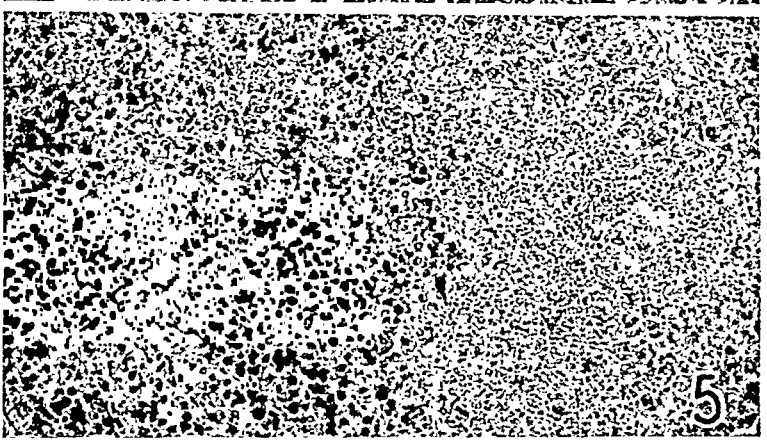
In Case 34 the liver showed grossly no difference between its halves. However, sections stained for iron showed a very conspicuous difference, similar to that seen in Case 6 (Fig. 6). This infant died at the age of two days with erythroblastosis fetalis and Kernicterus. Microscopically, the lungs showed hyaline membranes in some bronchioles, aspirated cornified cells, and pneumonia. Many petechial hemorrhages were present in various parts of the body. Esophagitis and gastric erosions were found, as well as foci of necrosis in the wall of hepatic arteries.

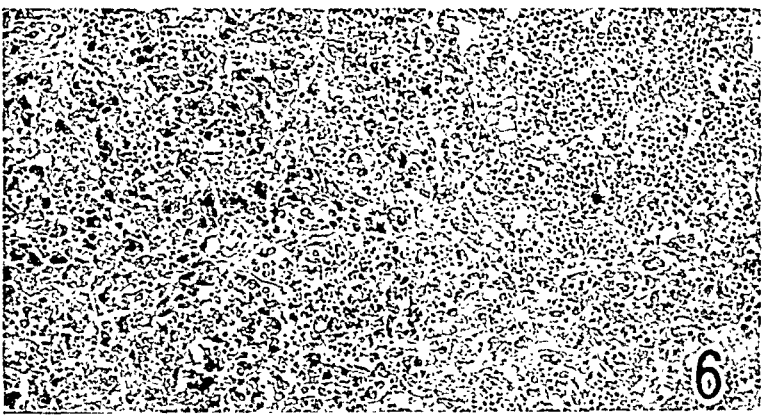
In Case 35 the infant had a typical history of severe asphyxia. The clinical diagnosis of partial separation of the placenta was made during labor and was later confirmed by examination of the placenta. The infant had to be resuscitated for two hours by artificial respiration, injection of coramine and aspiration of the respiratory passages. It went gradually downhill and expired two days after birth. Findings at autopsy included severe general congestion, focal hemorrhages in the epicardium, pleura, thymus, peritoneum and endometrium, areas of necrosis in the wall of the coronary and hepatic arteries, aspiration of cornified cells and pneumonia, as well as focal necrosis and hemorrhage in the adrenals. The liver was yellowish in color and showed distinct lobulation. An inconspicuous borderline between its halves was seen about 1.5 cm. to the right of the falciform ligament; the tissue to its right was more yellow in color than that to its left. Microscopically, the liver cells in the right half contained many more fat-free vacuoles and much more iron than those in the left half.

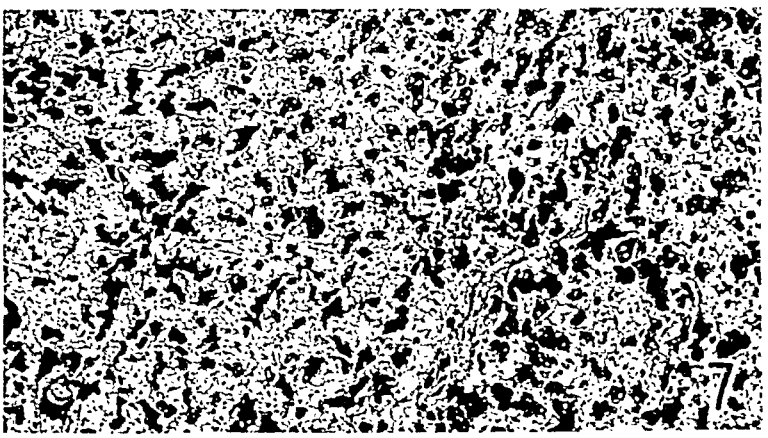
An example of the findings in older infants is furnished by an eight-day-old male infant (Case 40). His birth weight was 2700 grams. The onset of respiration was not spontaneous and stimulation was necessary. The baby whined during the first four days and never cried properly. He was restless and took only part of each feeding. Bowel movements had to be induced by enema. On the seventh day he was cyanotic and had feeble respirations for one hour. On the following day he was admitted to the hospital in poor condition and on this day, he died. At autopsy the body weighed 2160 grams, was edematous and jaundiced. The liver showed distinct lobulation, and a difference in the appearance of the

FIGS. 4 to 7. Sections showing the border of the right and left half of the liver. × 125. The right half is on the left side of each figure. Figures 4 (Case 12, hematoxylin-eosin) and 5 (Case 9, frozen section, Sudan III–hematoxylin) show much more fat in the right half than in the left. In Figure 6 (Case 6, Prussian blue reaction and basic fuchsin) there is an excess of iron on the right side. In Figure 7 (Case 3) the liver cells in the right half are thinner than in the left half.



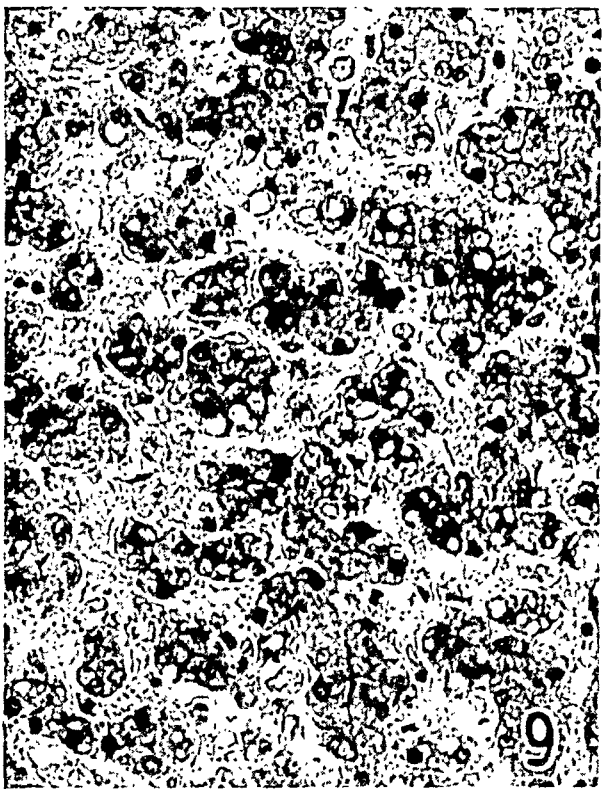






FIGS. 4–7





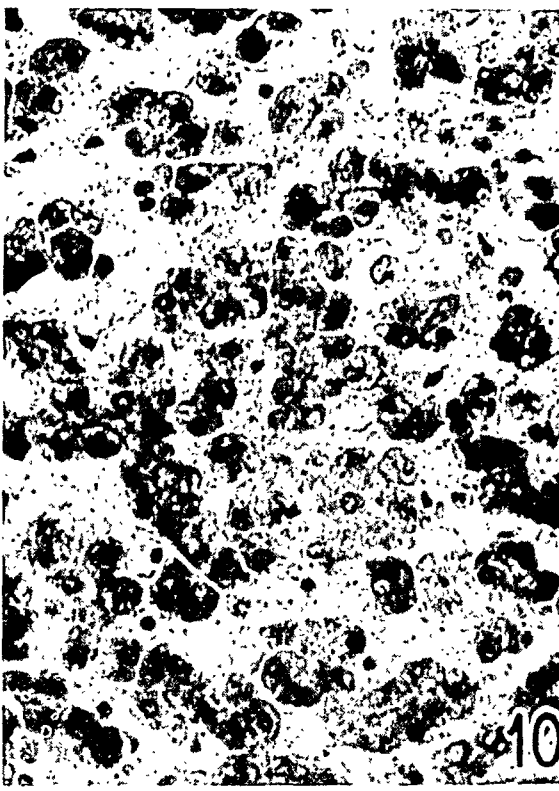
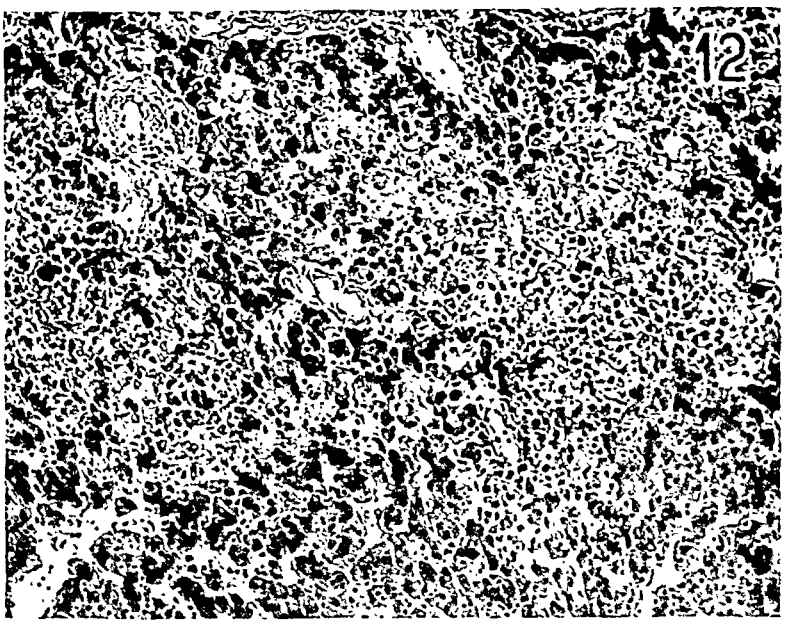


FIG. 8. Case 10. Gross appearance of liver, showing difference between their halves.
FIGS. 9 and 10. Case 24. Views of liver tissue from both halves. The right half (Fig. 9) shows a more granular cytoplasm with fatty and fat-free vacuoles. × 400.

halves as shown in Figure 13. Microscopically, the right half contained much fat and the left half very little. One might assume that owing to the poor condition of this infant throughout the eight days of its life, the degeneration present in the right half of the liver at birth was never reversed.





FIGS. 11 and 12. Case 25. There was a conspicuous difference in the gross appearance of the halves of the liver (Fig. 11). The right half was pale yellow and contained hemorrhagic areas. Microscopically, it showed many areas of necrosis (Fig. 12. × 125).

DISCUSSION

In evaluating the present findings one is reminded of the fact that in the adult the blood from certain tributaries of the portal vein enters one side of the liver, and the blood from other veins supplies the other side of the organ (Kinney and Ferrebee[6]). This explains the distribution of metastatic carcinoma or abscesses in certain cases. However, diffuse alterations of the parenchyma in one-half of the liver have, to the best of my knowledge, been reported only in experimental

animals but not in man. There is no evidence of the existence of any agent which might enter the portal vein of the fetus from just one of its tributaries, and then damage the cells in one-half of the liver. In search of an explanation of the present observations it was recognized that certain peculiarities of the fetal circulation might affect the liver; this was suggested by the fact that in the great

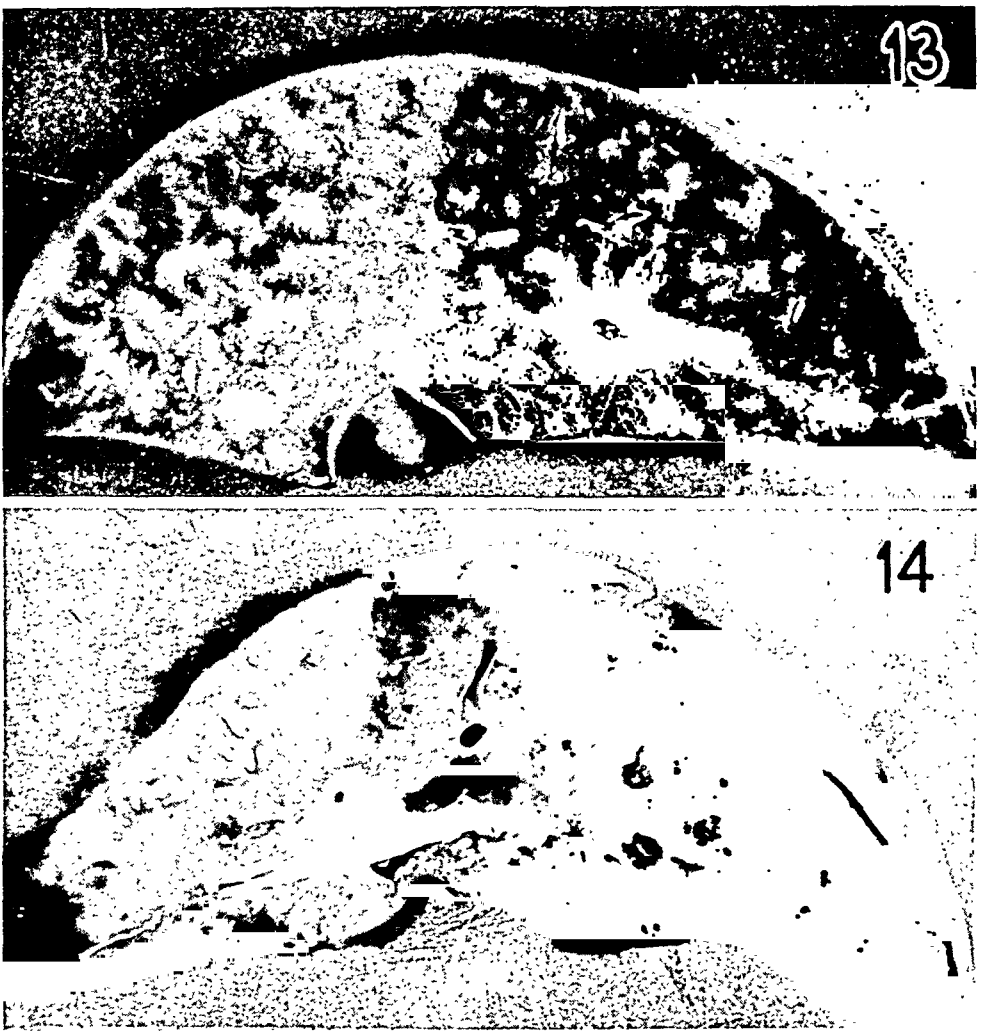


FIG. 13. Case 40. Grossly visible difference between the halves of the liver.
FIG. 14. Liver of a newborn infant after injection of diluted India ink into the umbilical vein. The ink has entered the tissue only on the left side (right side of figure).

majority of our cases the subjects were stillborn or newborn infants who might have acquired the abnormality *in utero*.

Six normal-appearing livers from autopsies on newborn infants were used in pertinent experiments. After clamping of the ductus venosus, diluted India ink was injected into the umbilical vein. It entered only the tissue of the left half of the liver and produced a dividing line which closely resembled that in the pathologic material (Fig. 14). This suggests that in the living fetus some of the

arterial blood of the umbilical vein may enter the left half, and not the right half, of the organ. This is in agreement with presentations of the development of the umbilical vein in text books of embryology. According to these, the degeneration in the embryo of certain parts of the paired umbilical veins leaves an asymmetrical state, and in one text book a diagram (Patten,[7] Fig. 406, D) clearly shows branches of the umbilical vein extending only into the left half of the liver.

The present pathologic findings suggest that the arterial blood of the umbilical vein has a protective action on the left half of the liver. Several lines of evidence suggest that this happens in anoxia before or at birth. Gillman and Gillman[2] have studied the effects of various types of anoxia on the liver, and have found the same changes which are present in our material, except for deposits of iron-containing pigment. The same authors found these changes in 18 of 25 stillborn infants examined. This has been confirmed in numerous autopsies performed during the period of collection of the present material; in many instances there was no difference between the halves of the liver. Table 2 gives data on signs of asphyxia found in the present material. In addition to the usual signs, such as focal hemorrhages in the thoracic viscera or the brain, or massive aspiration of vernix caseosa, two others are included which are frequently associated with the lesions under consideration, and have tentatively been correlated with asphyxia at birth. One of these is focal necrosis in the wall of arteries of the heart and liver, which occurs in stillborns and newborns with signs of severe asphyxia.[4] The other pathologic condition is acute esophagitis found in infants of all ages;[5] there are indications that it is a "shock" lesion (Penner and Bernheim[8]). Among newborn infants those with a history or with signs of severe asphyxia appear to be predisposed to esophagitis, owing perhaps to shock caused by asphyxia.[5]

In evaluating the incidence of arterial necrosis in the present series of cases, one should note that it is higher than in unselected autopsies (Table 2). Data for the latter are taken from previous work on coronary arteries,[4] corrected for additional cases with necrosis in hepatic vessels. There is no difference between the halves of the liver in the occurrence of arterial necrosis in the portal fields. This is readily accounted for by the fact that the blood supply of the arterial wall arises from branches of the arteries, and not from channels which may or may not carry blood from the umbilical vein.

Esophagitis is uncommon in stillborn infants, and mild when it occurs. All data in Table 2 referring to this lesion concern liveborn infants only. The much higher incidence in the present group is obvious.

The fact that only some of the infants with anoxic changes in the liver show a difference between the halves, suggests that protection by blood of the umbilical vein is effective only in a limited range of intensity or duration of anoxia.

The clinical histories reveal in many of the present cases the existence of prolonged periods of anoxia after birth. This may be related to the present findings either because it is a continuation (and therefore an indication) of previous intrauterine asphyxia, or because it may act on a liver the right half of which is more susceptible because of previous damage *in utero*.

A lateral difference in the distribution of iron-containing pigment is not readily explained. Large amounts of this pigment were present only in instances with erythroblastosis fetalis. It is conceivable that liver cells damaged by anoxia, or by a combination of anoxia and the effects of erythroblastosis may more readily absorb the pigment, or that the concentration of the latter in the blood stream is lower where there is admixture of blood coming direct from the placenta. There may be a cumulation of the effect of anoxia not only with erythroblastosis, but also with other diseases affecting the liver, *e.g.*, in Case 33 with an undetermined, presumably infectious condition (perhaps toxoplasmosis or fungus infection affecting almost every organ in the body).

Four older infants exhibited lateral differences in the liver similar to those in newborns (see the third group of cases in Table 1). Whether or not this is a condition persisting from the time of birth, cannot be decided with assurance.

TABLE 2

INCIDENCE OF FINDINGS SUGGESTIVE OF ASPHYXIA IN NEWBORN INFANTS WITH DEGENERATION OF THE LIVER PREDOMINANT IN THE RIGHT HALF*

GROUP OF CASES	NUMBER OF CASES	ASPIRATION OF VERNIX CASEOSA	PETECHIAL HEMORRHAGES	NECROSIS IN CORONARY OR HEPATIC ARTERY	ESOPHAGITIS, GASTRIC EROSIONS
With degeneration in right half of liver:					
Stillborn	20	13 of 18	15 of 15	2 of 18	
Liveborn, up to 3 days	18	8 of 18	14 of 18	8 of 18	12 of 16
Total	38	21 of 36	29 of 33	10 of 36	
Control: unselected cases				26 of 221	18 of 86 (liveborn only)

* Since information on each of these points was not available in every instance (see Table 1), the number of cases examined varies and is specified for each condition tabulated.

This explanation is readily acceptable in Cases 42 and 44 in which a difference in the amount of hemosiderin in the tissue may well have persisted for several weeks. In 3 of the 4 older infants the difference concerns the fat content. In one instance (Case 43), there were foci of necrosis in the right half of the liver; they did not appear to have been quite recent and may or may not have dated from birth. As was mentioned before, an increased susceptibility of the right half of the liver to damage may conceivably persist for some time following intrauterine asphyxia, so that postnatal influences may have a greater effect on that part of the organ. The absence of concomitant signs of neonatal asphyxia in these cases (Table 1) is obviously due to the long time elapsed since birth.

COMMENT

Findings of a difference in the structure of the lateral halves of the liver in infants are significant as an indication of prenatal liver damage, presumably due to anoxia occurring while the placental circulation was intact. In liveborn infants this finding may be helpful in the evaluation of signs of anoxia in relation

to the time of birth. However, it should be remembered that not all cases of intrauterine asphyxia show this feature; on the other hand, a difference between the halves of the liver may be increased after birth by the action of anoxia or other injurious agents on the previously damaged right half.

Knowledge of this condition is also desirable because the finding is conspicuous in some instances and the pathologist may wish to interpret it; he may then make the diagnosis of intrauterine asphyxia at the time of autopsy, before sections are available. In macerated stillborn infants this condition may occasionally be recognized when the preservation of tissues is too poor for satisfactory microscopic examination.

It is conceivable that a similar difference, but with more severe changes on the left side, may result from injurious agents reaching the fetus from the umbilical vein; no such case has yet been observed.

SUMMARY

In about 15 per cent of infants less than two months of age degenerative changes of various degrees were found in the liver, more severe in the right half than in the left. Most of these infants were stillborn or died within three days of birth.

It was demonstrated that a similar difference between the halves of the liver exists with regard to its circulation, as blood from the umbilical vein reaches the parenchyma of the left half only. This suggests that *in utero* the arterial blood of the umbilical vein presumably protects the left half of the liver from anoxia.

Other findings in the present cases support the assumption that the infants suffered severe anoxia.

The presence of a difference in the appearance of the lateral halves of the liver, with a sharp dividing line somewhat to the right of the falciform ligament, therefore, suggests degeneration due to intrauterine anoxia.

REFERENCES

1. Ghon, A.: Leber, Gallenblase und Gallenwege, Pancreas. In: L. Aschoff: Pathologische Anatomie. Vol. 2. Jena: Gustav Fisher, pp. 798–868, 1936.
2. Gillman, J., and Gillman, T.: Anoxia and the liver with special reference to shock and chronic malnutrition. South African J. M. Sci., **13**: 11–43, 1948.
3. Gruenwald, P.: Rupture of liver and spleen in the newborn infant. J. Pediat., **33**: 195–201, 1948.
4. Gruenwald, P.: Necrosis in the coronary arteries of newborn infants. Am. Heart J., in press.
5. Gruenwald, P., and Marsh, M. R.: Acute esophagitis in infants; pathologic observations. In preparation.
6. Kinney, T. D., and Ferrebee, J. W.: Hepatic abscess; factors determining its localization. Arch Path., **45**: 41–47, 1948.
7. Patten, B. M.: Human Embryology. Philadelphia: The Blakiston Company, Fig. 406D, p. 645, 1946.
8. Penner, A., and Bernheim, A. I.: Acute postoperative esophageal gastric and duodenal ulcerations; further study of the pathologic changes in shock. Arch Path., **28**: 129–140, 1939.

ADHESIVENESS OF BLOOD PLATELETS IN THROMBOEMBOLISM AND HEMORRHAGIC DISORDERS

II. Diagnostic and Prognostic Significance of Platelet Adhesiveness*

SYLVAN E. MOOLTEN, M.D., LEO VROMAN, and GEORGINE M. S. VROMAN

From the Research Division, Laboratory Service, St. Peter's General Hospital, New Brunswick, New Jersey

In the preceding paper [14] a method was described in which a wick of glass-wool is employed as an adsorbing filter to measure platelet adhesiveness in a small sample of citrated blood. Extended trial of the method has given satisfactory evidence of its value in analyzing conditions in which altered behavior of blood platelets is a significant factor. The present report is concerned with the diagnostic and prognostic significance of platelet adhesiveness in various pathologic conditions.

HEMORRHAGIC DISORDERS

Thrombocytopenic purpura. In 3 cases of thrombocytopenic purpura extreme depression of platelet adhesiveness was found (Table 1, A). Two were cases of idiopathic thrombocytopenic purpura, the third a case of splenomegalic purpura complicating cirrhosis. In 3 cases of secondary thrombocytopenic purpura resulting from widespread leukemic involvement of the bone marrow, platelet adhesiveness was generally elevated despite the low total count. The contrast between the two types of purpura is possibly significant theoretically as evidence of the difference in their underlying mechanism. Thrombocytopenic purpura which is caused by hypersplenism, as may be the case in the first 3 patients, has been explained as the result of the suppressive action of the splenic hormone, *thrombocytopen*,[27] upon megakaryocytes. Its effect is manifested by diminished segmentation of the cytoplasm of the megakaryocytes into platelets.[4] The reduced adhesiveness found by our method in platelets formed from such megakaryocytes suggests the continuing action of thrombocytopen upon platelets after they enter the circulation. This supposition is partly confirmed by our finding that thrombocytopen, added to blood *in vitro*, will depress the adhesiveness of platelets having initially normal or high adhesiveness.[15] Baar [1, 2] has shown that the platelets of patients with purpura also manifest greatly retarded lysis *in vitro* in contrast to those of hemophilia or other conditions. The normal or elevated platelet adhesiveness noted by us in thrombocytopenic purpura secondary to leukemia probably indicates that the megakaryocytes in this condition are much reduced in number, yet are capable of segmenting in a normal manner in response to the increased demand for platelets. Similar findings were encountered in aplastic anemia.

* Aided by grants from the Dazian Foundation, Dr. F. M. Clarke, and Mr. Harry R. Hillman.

Received for publication, May 13, 1949.

Effects of thrombocytosin. The action of thrombocytosin (obtained from egg yolk[13]) proved to be virtually the exact opposite of that of thrombocytopen (see

TABLE 1

BLEEDING TIME (IVY[11]), TOTAL PLATELET COUNT, ADHESIVE INDEX, AND ADHESIVE PLATELET COUNT IN PATIENTS WITH HEMORRHAGIC DISORDERS. EFFECTS OF THROMBOCYTOSIN

	BLEEDING TIME (IVY)	TOTAL PLATELETS	INDEX	ADHESIVE PLATELETS
A. Thrombocytopenic Purpura				
Essential thrombycotopenic purpura (2 cases)				
1. Initial study	>25′	51,000	0.78	4,000
17 hr. after Thrombocytosin	8′ 20″	42,000	1.47	20,000
2. Initial study	>30′	31,000	0.67	0
6½ hr. after Thrombocytosin	15½′	31,000	1.15	9,600
Purpura associated with cirrhosis				
Initial study	15½′	135,000	0.69	0
4 hrs. after Thrombocytosin	6′ 40″	135,000	1.26	49,000
17th day: exitus				
Purpura associated with leukemia (3 cases)				
1. Initial study	>15′	33,000	1.71	17,000
4 weeks later (remission)	6′	346,000	1.48	153,000
2. Initial study	9′ 50″	120,000	1.53	55,000
13th day	15′ 20″	36,000	1.05	10,000
20th day: after Thrombocytosin	>25′	34,000	1.29	12,000
22nd day: after Thrombocytosin	>15′	193,000	2.35	120,000
40th day	>15′	44,000	1.28	19,000
81st day: exitus				
3. Single test	15′	22,000	2.08	13,000
Purpura associated with aplastic anemia	>20′	35,000	1.86	19,000
B. Nonthrombocytopenic Purpura				
Purpura simplex (2 cases)				
1. Initial study	2′ 8″	218,000	0.74	8,000
3 mo. later: after calcium	2′ 10″	220,000	1.23	77,000
2. Single test	2′ 15″	271,000	0.85	35,000
Pseudohemophilia				
Initial study	2′ 50″	153,000	0.88	23,000
2 mo. later	19′ 15″	388,000	1.27	141,000
Avitaminosis C	11′	224,000	1.17	71,000
C. Hemophilia				
1. Coagulation time 35 minutes	8′	297,000	1.16	94,000
2. Coagulation time 55 minutes		278,000	1.68	138,000
3. Coagulation time 12 minutes	5′ 50″	280,000	1.08	76,000

below) in respect to its effects on platelet count, platelet adhesiveness, and bleeding tendency. In 4 of the cases of purpura cited above, thrombocytosin injected intramuscularly caused prompt rise in platelet count and adhesiveness and striking shortening of bleeding time (Table 1, A). Failure of the total platelet

count to rise significantly after thrombocytosin despite rise in adhesiveness and amelioration of bleeding tendency was common. Possibly the rise was missed because of the speed of utilization of newly formed adhesive platelets.

Non-thrombocytopenic purpura. In a few cases of non-thrombocytopenic purpura with normal clotting mechanism (Table 1, B), no consistent relationship was established between bleeding time and platelet adhesiveness, although at times both deviated significantly from normal. Insofar as bleeding time can be considered a measure of capillary function, the apparent lack of direct dependence of the latter upon platelet adhesiveness may indicate that the ultimate basis of the disorder lies within the protoplasmic elements of platelets and endothelial cells as a common biologic defect. In at least 2 cases dietary deficiency was a possible cause. The relation of such cases to "pseudohemophilia" is not clear but may be worthy of study.

Hemophilia. In 3 cases of hemophilia platelet adhesiveness was found to be normal or slightly elevated during periods of active bleeding prior to transfusion and other therapy (Table 1, C). These data are consistent with other observations indicating that the blood platelets of hemophiliacs do not differ intrinsically from those of normal subjects and owe their apparent differences to the clotting anomaly which resides in the plasma (Baar and Székely,[2] Patek and Stetson,[20] Quick[21]). The diminished rate of platelet loss from hemophilic blood in the rotating tube test for platelet adhesiveness (Wright[34]), advanced as evidence of low platelet adhesiveness, may therefore be interpreted as delayed lysis[1] as the result of retarded thrombin formation[21].

Infectious reticulo-endotheliosis. Moderate hypo-adhesiveness, with or without corresponding thrombocytopenia, was found in generalized nonsuppurative infections (reticulo-endothelioses) associated with splenomegaly, such as infectious mononucleosis and chronic brucellosis (Table 2, A) and can be reasonably predicted for other hypersplenic conditions in this category which are sometimes complicated by thrombocytopenic purpura (as in Felty's syndrome, miliary tuberculosis, sarcoidosis, typhoid fever and kala-azar[16]).

Hyperthyroidism; pregnancy. A tendency to hypo-adhesiveness of platelets was also noted in hyperthyroidism (Table 2, B) and in late pregnancy preceding delivery (Table 2, C). Shortly after delivery many giant platelets were found in the circulation in several puerperal women. A steep rise in platelet count occasionally followed thyroidectomy.

PLATELET HYPERADHESIVENESS AND THROMBOEMBOLIC DISEASE

Surgical and accidental trauma. The increased adhesiveness of platelets noted by Wright[33] in surgical patients in the first two or three weeks postoperatively was demonstrable also by the glass-wool filter test (Table 3, A). In many instances a slight rise appeared within the first hour or two after operation, followed on the next day by moderate fall in platelet count and adhesiveness, which was suggestive of increased utilization of platelets in formation of wound thrombi. Beginning three to five days after operation the total count and adhesiveness generally rose progressively, reached a peak, and gradually fell to normal. Similar

findings were recorded after accidental trauma, especially cases of fracture. Exceptions to this pattern of platelet behavior were encountered not infrequently and may be interpreted as suggestive evidence of complication, particularly silent thrombosis (see below).

Ischemic necrosis (myocardial infarction, pulmonary infarction, gangrene). In acute myocardial infarction platelet adhesiveness rose quickly after the onset and often aided clinical evaluation of symptoms in advance of positive electrocardiographic findings (Table 3, B). The total platelet count was often elevated as well, although not necessarily in corresponding degree. As experience with the

TABLE 2

DIMINISHED PLATELET ADHESIVENESS WITHOUT MANIFEST HEMORRHAGIC TENDENCY

	TOTAL PLATELETS	INDEX	ADHESIVE PLATELETS
A. Systemic Non-Suppurative Infections With Splenomegaly (Reticulo-endothelioses)			
Infectious mononucleosis			
Initial study	66,000		
2 days later	146,000	0.91	26,000
Chronic brucellosis	251,000	1.00	58,000
B. Hyperthyroidism			
Graves' disease	240,000	0.96	49,000
Graves' disease	185,000	1.22	62,000
Nodular goiter	531,000	0.8	48,000
Menopausal hyperthyroidism	284,000	1.04	72,000
C. Pregnancy At Term			
Normal pregnancy, ante partum	253,000	0.90	42,000
Normal pregnancy, ante partum	213,000	0.97	45,000
Normal pregnancy, ante partum	344,000	0.96	71,000
Normal pregnancy, ante partum	182,000	1.2	61,000

test grew, increasing reliance was placed on the measurement of platelet hyperadhesiveness in determining the indications for heparin or dicumarol therapy in coronary arterial disease.

Similar findings were encountered following pulmonary infarction and occlusion of the arterial supply to an extremity. The impression was gained that recurrence or propagation of thrombi occurred more quickly than otherwise, as a sequel to such rise in platelet count and adhesiveness. The latter probably accounts to some extent for the secondary venous thrombosis in ischemic gangrene of an extremity, as found commonly in the venae comites of occluded arteries in the amputated specimen.

Dermatitis; cellulitis. In cases of extensive cutaneous or subcutaneous inflammation, platelet count and adhesiveness generally mounted considerably (Table 3, C).

Dermatitis and cutaneous irritation by chemical or physical agents have long been known to elevate the platelet count (Steiner and Gunn[26]). Conceivably thrombocytosin is mobilized from subcutaneous fat by such means. The same

TABLE 3

PLATELET HYPERADHESIVENESS

	TOTAL PLATELETS	INDEX	ADHESIVE PLATELETS
A. Convalescence Following Trauma			
Surgical procedures			
Hysterectomy, 1½ hr. postoperative	325,000	1.96	184,000
Herniorrhaphy, 3rd day	282,000	2.3	176,000
Caesarean section, 2nd day	323,000	1.46	140,000
Herniorrhaphy, 3rd day	440,000	1.94	244,000
Thyroidectomy, 6th day	615,000	1.06	162,000
Mastectomy (carcinoma), 8th day	289,000	1.9	157,000
Colonic resection (carcinoma), 12th day	558,000	1.26	202,000
Colonic resection (carcinoma), 15th day	493,000	1.36	197,000
Accidental trauma			
Compound fracture, leg, 5th day	342,000	2.42	215,000
Vertebral fracture, 16th day	337,000	4.02	259,000
B. Infarction and Gangrene			
Myocardial infarction			
1. 12 hr. after onset	302,000	2.17	180,000
2. 3rd day after onset	344,000	1.26	124,000
3. 4th day after onset	686,000	2.32	425,000
4. 11th day after onset	420,000	1.14	129,000
Pulmonary infarction			
Initial study, 3rd day	431,000	1.53	195,000
14th day	656,000	1.18	213,000
Peripheral arterial closure			
Thrombosis, femoral artery	664,000	1.12	198,000
Embolism, popliteal artery	416,000	1.41	173,000
Thrombosis, popliteal artery	564,000	1.26	202,000
Ligation of femoral artery	346,000	1.79	180,000
C. Inflammation of Skin and Subcutis			
Exfoliative dermatitis	737,000	4.17	573,000
Generalized erythroderma	446,000	1.81	235,000
"Cellulitis" of foot	358,000	1.52	160,000
D. Carcinomatosis (Unoperated)			
Carcinoma of ascending colon	315,000	1.55	145,000
Carcinoma of sigmoid; metastases	391,000	3.57	290,000
Carcinoma of rectum; metastases	340,000	1.68	170,000
Carcinoma of cervix	602,000	3.48	443,000
Carcinoma of lung	320,000	2.71	214,000
Carcinoma of lung	400,000	2.18	240,000

TABLE 3—*Concluded*

	TOTAL PLATELETS	INDEX	ADHESIVE PLATELETS
E. Blood Dyscrasias of Erythroleukemia Group (Idiopathic Thrombocythemia, Polycythemia Vera, Essential Thrombophilia)			
Idiopathic thrombocythemia, 3 mo. after splenectomy	2,235,000	3.93	1,705,000
Idiopathic thrombocythemia with leukemoid reaction	702,000	1.38	282,000
Polycythemia vera	528,000	3.27	380,000
Essential thrombophilia	433,000	3.53	320,000
Essential thrombophilia	556,000	4.80	444,000
Essential thrombophilia	493,000.	2.45	314,000
F. Post-Hemorrhagic Thrombocytosis			
Polycythemia vera: phlebotomies			
Initial study, 3rd day	648,000	5.42	535,000
7th day	1,080,000	2.39	680,000
9th day	1,224,000	10.9	1,136,000
Gastric ulcer; 15th day after massive hematemesis	466,000	2.72	312,000

explanation has been given previously for the rise in platelet count following operation.[13]

Cancer. Persistent hyperadhesiveness of platelets was a regular finding in cancer, generally out of proportion to the level of platelet count, which was only moderately elevated or normal. Peripheral venous thrombosis was observed in several of the cases, unrelated to surgical intervention. The tendency to thrombosis in cancer, long familiar to clinicians (Trousseau's sign) has not been adequately explained. High platelet count has been recorded in a relatively small proportion of cases[17] and cannot in itself be satisfactorily correlated with the tendency to thrombosis. Increased platelet adhesiveness in cancer not only suggests a clue to the mechanism of thrombosis in such cases but provides a sign which may be helpful in the differential diagnosis of suspected cancer and in the detection of occult cancer.

Intrinsic platelet disease (idiopathic thrombocythemia, polycythemia vera, essential thrombophilia). Abnormalities in platelet behavior, unrelated to significant change in platelet count, have been claimed as primary hematologic entities in certain hemorrhagic disorders, such as hereditary or acquired "pseudohemophilia" (von Willebrand and Jürgens[31]) and congenital hemorrhagic "thrombocytic dystrophy" (Bernard and Soulier[3]). An intrinsic defect in platelets may also account for the hemorrhagic tendency in idiopathic thrombocythemia ("hemorrhagic thrombocythemia"[6, 18, 22, 30] despite remarkably high platelet counts and tendency to recurrent thrombosis.[23, 24] In one case of this type platelet adhesiveness and total count were markedly elevated (Table 3, E), yet prolongation of bleeding time and excessive tendency to bruise were present and capillary microscopy revealed abnormally wide and tortuous vascular loops in the nail-fold. The defect in hemostasis may depend on other platelet factors than adhesive-

ness, possibly deficiency in platelet vasoconstrictor substance.[35] In this patient the thrombotic tendency had led previously to closure of the splenic vein, and the ensuing painful splenomegaly was treated by splenectomy. In a second case of idiopathic thrombocythemia thrombosis of the femoral artery resulted in gangrene, necessitating amputation of the limb. In this patient no abnormality, past or present, was observed in the erythrocyte picture and no hemorrhagic tendency appeared. In the latter part of the clinical course a leukemoid change developed (95,000 leukocytes per cu. mm., chiefly immature) and at autopsy the marrow exhibited pronounced nodular hyperplasia of the myeloid elements but no signs of leukemia. The relationship of these cases to polycythemia vera (erythremia) and the more inclusive category of erythroleukemia (Di Guglielmo[5]) seems likely.[18, 24]

In a patient with polycythemia vera a series of arterial and venous thrombotic episodes had occurred. Platelet count and adhesiveness, which had been consistently much elevated above normal, rose to extremely high levels following daily withdrawal of 50 ml. of blood for eight successive days (Table 3, F). A hemorrhagic tendency was also present in this case, taking the form of massive painful subcutaneous and intramuscular hematomas following minor trauma.

Three patients, all males, with recurrent thrombotic disease were studied; in all of them platelet adhesiveness persisted at high levels for long periods of observation. Total platelet counts were only moderately elevated. Bleeding time tended to be shorter than normal. In the absence of evident cause for platelet hyperadhesiveness, such as cancer or chronic infection, these patients were classed as having "essential thrombophilia" (Nygaard and Brown[19]). One patient had suffered from cerebral and coronary arterial thromboses and repeated attacks of pulmonary infarction. In a second patient multiple pulmonary emboli resulted in acute cor pulmonale; later he suffered from thrombosis of the axillary artery and probable renal infarction. The third patient, a man of 22, developed gangrene of one leg and had a history of venous thrombosis of the other leg. White and red thrombi of various ages were found occluding the arteries and veins of the limb after amputation, but no significant changes were discovered in the walls of these vessels except those which appeared secondary to the thrombi.

Post-hemorrhagic thrombocytosis. Regenerative thrombocytosis, as seen following hemorrhage, is accompanied by increased platelet adhesiveness. The latter may thus account for the heightened tendency to thromboembolism in such instances, as in the case of gastric ulcer complicated by massive hematemeses listed in Table 3 (F) and in the case of polycythemia vera mentioned above. The increased danger of thrombosis following phlebotomy[8] or administration of phenylhydrazine[7] in polycythemia vera has brought about an attitude of caution regarding their indiscriminate use.

INFLUENCE OF THROMBUS FORMATION ON PLATELET COUNT AND ADHESIVENESS

In several instances of proved thrombotic disease opportunity was provided for study of the levels of platelet count and platelet adhesiveness over an extended period by means of serial tests performed at intervals of one to four days. In most

of these cases parallel studies were made of coagulation factors, particularly prothrombin activity (Quick method), heparin tolerance *in vitro*,[29] and clot retraction time.[9] The curves of rise and fall of these factors in the circulating blood yielded much information on the relative significance of the hematic changes which accompany the formative (preclinical) phases of thrombosis and the effects of heparin, dicumarol, and thrombocytopen.

In general, rapid fall in platelet count gave warning of massive thrombus formation. Our findings in this respect parallel those of Kristenson,[12] Normann,[12] Kojima,[12] and Rosenthal,[23] who have set forth the diagnostic value of sudden fall in platelet count in the preclinical recognition of thrombosis. Drop in adhesive platelets proved to be much more decisive as a diagnostic sign than drop in total platelet count. It appears reasonable to interpret this phenomenon as signifying the selective withdrawal of many "sticky" platelets from circulation during the building up of a white thrombus in some portion of the circulatory tree (Table 4, A).

Coronary thrombosis. In cases of coronary thrombosis, fall in platelet adhesiveness and total platelet count suggested the growth of mural thrombi in the left ventricle and the consequent threat of peripheral embolism (Table 4, A). Similar significance could be attached to significantly diminished platelet adhesiveness as an initial finding in place of the elevated adhesiveness ordinarily found (Table 4, B).

Cancer. In thrombophilic states such as cancer, in which the proportion of adhesive platelets to the total platelet count is particularly high, thrombus formation may, therefore, impoverish the circulation of blood platelets to a striking degree. In the case of a patient suffering from gastric carcinoma such rapid platelet withdrawal resulted in true thrombocytopenic purpura (Table 4, B) and marked hemorrhage from the tumor. Autopsy disclosed massive phlebothrombosis of both lower extremities, thrombosis of the right auricular appendage, nonbacterial thromboendocarditis of the aortic valve ("cachectic endocarditis"), and multiple embolic lesions in the lungs, the brain, and several toes of each foot. Metastases of the tumor were relatively few and did not involve the bone marrow.

Silent thrombosis. In a number of cases, surgical and nonsurgical, significant fall in total and adhesive platelet count was unassociated with clinically apparent thrombosis and the patients convalesced uneventfully. This result was not wholly unexpected in the light of published pathologic studies demonstrating the marked discrepancy which exists statistically between clinically recognized deep venous thromboses and their far higher actual incidence revealed by autopsy.[10]

ACTION OF ANTICOAGULANTS AND THROMBOCYTOPEN

Heparin. In doses of 100 mg. or more intravenously, heparin caused prompt lowering of platelet adhesiveness (Table 4, C). Similar effects were observed when heparin was injected subcutaneously in sites pretreated with hyaluronidase to facilitate absorption.[28] Smaller doses had little or no effect.

A characteristic finding in patients given heparin was the opposite behavior of platelet count to that of platelet adhesiveness. In serial tests the respective

TABLE 4.

INFLUENCE OF THROMBUS FORMATION, ANTICOAGULANT THERAPY AND THROMBOCYTOPEN UPON TOTAL PLATELET COUNT, ADHESIVE INDEX, AND ADHESIVE PLATELET COUNT

	TOTAL PLATELETS	INDEX	ADHESIVE PLATELETS
A. Pre-Clinical and Clinical Phases of Thrombosis			
Phlebothrombosis			
1. Initial study	431,000	1.53	195,000
3rd day	335,000	1.04	85,000
6th day	256,000	0.95	51,000
8th day: pulmonary infarction			
2. Initial study	444,000	4.33	349,000
8th day	215,000	2.03	121,000
13th day: fatal pulmonary embolism			
3. Initial study	300,000	1.38	123,000
4th day: severe chest pain			
5th day	220,000	0.91	39,000
Myocardial infarction associated with mural thrombi			
1. Initial study	302,000	2.17	180,000
5th day	186,000	1.03	46,000
11th day: sudden exitus			
2. Initial study	686,000	2.32	425,000
2nd day	296,000	1.12	86,000
20th day: sudden abdominal pain			
B. Post-Thrombotic Depletion of Adhesive Platelets			
Myocardial infarction			
1. 1 week after onset	193,000	1.06	51,000
2. 1 week after onset	295,000	0.87	44,000
3. Patient moribund	196,000	1.08	54,000
Multiple thrombotic lesions associated with purpura in patient with gastric carcinoma			
Initial study	138,000	0.92	25,000
2nd day	96,000		
5th day	62,000		
C. Action of Heparin			
Myocardial infarction			
Initial study	337,000	1.80	177,000
3rd day: heparin 150 mg. q. 4 hr.			
6th day	391,000	1.11	115,000
Compound fracture			
Initial study	377,000	1.2	126,000
4th day, after 100 mg. heparin	462,000	0.65	0
7th day, after total of 900 mg.	400,000	0.74	15,000
12th day	512,000	0.87	74,000
17th day	416,000	1.32	159,000
Gangrene following ligation of femoral artery			
Initial study, after 1400 mg. heparin	228,000	1.93	126,000
3rd day	346,000	1.79	180,000
7th day	375,000	1.05	98,000
10th day	742,000	0.72	17,000
14th day	402,000	1.27	146,000

TABLE 4—*Concluded*

	TOTAL PLATELETS	INDEX	ADHESIVE PLATELETS
D. Action of Dicumarol			
Postoperative thrombophlebitis (prothrombin <10%)	362,000	0.91	65,000
Femoral artery ligation, 3rd day (prothrombin <10%)	196,000	0.97	42,000
Myocardial infarction, 9th day (prothrombin <10%)	235,000	2.93	163,000
Myocardial infarction, 7th day (prothrombin <10%)	277,000	1.27	101,000

E. Action of Thrombocytopen

	BLEEDING TIME (IVY)	TOTAL PLATELETS	INDEX	ADHESIVE PLATELETS
Essential thrombophilia				
Initial study	1′ 30″	377,000	1.94	209,000
13 hr. after 75 mg. Thrombocytopen	1′ 30″	273,000	1.97	154,000
3rd day	3′ 10″	226,000	2.05	130,000
8th day	5′ 30″	249,000	1.11	72,000
13th day	2′ 10″	260,000	1.88	141,000
Essential thrombophilia				
1. First series				
Initial study	4′	378,000	1.66	185,000
6 hr. after 100 mg. Thrombocytopen	11′ 30″	451,000	1.09	126,000
2nd day	4′ 20″	264,000	0.86	36,000
4th day	7′	313,000	1.25	111,000
6th day	5′ 20″	378,000	1.41	158,000
2. Second series (same patient)				
Initial study	9′	278,000	1.67	136,000
1st day after 50 mg. Thrombocytopen	7′ 30″	229,000	0.82	25,000
2nd day	7′ 20″	247,000	0.93	47,000
8th day	4′ 50″	258,000	1.33	100,000
Arteriosclerotic gangrene, thrombosis of popliteal artery				
Initial study	5′	500,000	1.40	206,000
1st day after 150 mg. Thrombocytopen	10′ 10″	337,000	1.53	151,000
2nd day	6′	418,000	1.41	175,000

curves diverged, that of the total count tending to rise and that of platelet adhesiveness falling simultaneously in similar or greater degree. Cessation of heparin treatment was followed by reversal of these curves to pretreatment levels with slight transitory overshooting. These phenomena are probably best explained by the suppressive action of heparin on platelet lysis indirectly by curtailing formation of thrombin, the normal platelet lysin,[21] thus permitting longer survival of aging platelets in circulation. In one case the heparin effect developed slowly, beginning after treatment with heparin had been stopped because of hematuria after a total dose of 1400 mg. This delayed action of heparin with sharp but transitory peak about the 10th day suggested release of a large quantity of previously administered heparin from storage, possibly in mast cells, as observed by Schürer.[25]

Dicumarol. Hypoprothrombinemia induced by dicumarol had little effect on platelet adhesiveness except in a few instances in which prothrombin activity had been held to less than 10 per cent of normal for two or more days. In such instances the adhesive index was reduced to 0.9–1.0 but not below as with heparin (Table 4, D).

On several occasions rapid but transitory rise in prothrombin activity from relatively low levels to 90–100 per cent of normal or higher paralleled rapid elevation in both platelet count and platelet adhesiveness following acute tissue injury (*e.g.*, surgical operations, ischemic gangrene of a limb or pulmonary infarction). The circumstances of these findings were less suggestive of absolute increase in prothrombin concentration than of increased activity of accelerator factors affecting the rate of conversion of prothrombin to thrombin through increased platelet lysis.[16, 32] Further studies are needed to settle this point.

Thrombocytopen. This agent, originally shown by Troland and Lee[27] to exist in the crude acetone extract of the spleen of cases of thrombocytopenic purpura, has been observed by one of us[13] to be lipoid in nature and the product also of the normal spleen. On the premise that the normal spleen functions as a protective mechanism against thrombosis by its control of platelet production and adhesiveness, thrombocytopen (obtained from bovine spleen) was administered to several patients with increased platelet adhesiveness and total count. Fall in adhesiveness and total count for one or two days followed intramuscular injection of a saline suspension. A more prolonged effect occurred when it was injected dissolved in peanut oil or sesame oil. Thus in one case of essential thrombophilia, a sustained slight fall in total count and distinct fall in platelet adhesiveness were observed over a period of about two weeks with peak effect about the eighth day (Table 4, E). Similar effects of shorter duration were obtained in another patient with essential thrombophilia and in a patient with peripheral vascular disease with gangrene. Much more striking effects had been obtained previously in numerous experiments on rabbits, in which doses of 5–10 mg. caused pronounced drop in total count and adhesiveness, the effect lasting about three days after a single intravenous dose.[13, 15]

Certain data indicate that, in addition to its effect on megakaryocytes, thrombocytopen acts directly on blood platelets already formed and discharged into the circulation. In a large series of experiments[15] minute amounts of thrombocytopen (0.001–0.010 per cent) added to citrated human or rabbit blood *in vitro* were found repeatedly to reduce platelet adhesiveness in significant degree, as measured with the glass-wool filter, whereas highly purified cholesterol in equivalent amounts proved inert. On the other hand, thrombocytosin, the platelet-increasing factor found in adipose tissue and egg yolk, increased platelet adhesiveness under the same conditions, paralleling its action *in vivo*. Thrombocytopen thus appears to be the natural antagonist to thrombocytosin in respect to the platelet factors concerned not only in thrombosis but in the hemostatic mechanism in general.

SUMMARY

Measurement of platelet adhesiveness by the glass-wool filter may aid in diagnosis and interpretation of hemorrhagic and thrombotic disease. In thrombocy-

topenic purpura extremely low adhesiveness is suggestive of hypersplenism; normal or elevated adhesiveness implies primary marrow disease (leukemia, aplastic anemia). Moderate hypersplenism, revealed by reduced platelet adhesiveness, is common in non-suppurative systemic infections, hyperthryroidism, and late pregnancy. Suppression of thrombin formation by heparin, retarding platelet lysis, also reduces platelet adhesiveness; the total count, however, is maintained or slightly raised. Dicumarol has much less effect on adhesiveness. In hemophilia the adhesiveness is normal or elevated.

Hyperadhesiveness, with or without rise in total platelet count, accompanies cellular destruction anywhere in the body. It occurs regularly during uncomplicated convalescence from operation or accidental trauma. It is marked in cases of ischemic necrosis, *e.g.*, myocardial infarction and peripheral gangrene.

Continuous hyperadhesiveness of platelets is common in cancer and may aid in diagnosis of hidden cancer. It may also occur as part of a blood dyscrasia (polycythemia vera, idiopathic thrombocythemia) or as an independent entity (essential thrombophilia).

Marked platelet hyperadhesiveness predisposes to thrombosis. Rapid fall in platelet count, particularly adhesive platelets, warns of developing thrombosis, usually massive, as in a large vein or in the ventricle following myocardial infarction. In extreme cases of thrombotic disease, as in carcinoma or essential thrombophilia, the blood may be so rapidly depleted of adhesive platelets that purpura occurs.

Thrombocytosin, a fat-soluble agent stored in adipose tissue and abundant in egg yolk, increases platelet count and adhesiveness. It is probably mobilized by lytic enzymes during cellular breakdown or by direct injury of adipose tissue. It is also absorbed with the diet. Thrombocytopen, formed by the spleen, appears to be its antagonist. Both agents affect platelet count and adhesiveness significantly in man and may have therapeutic application, thrombocytosin in the treatment of purpura and thrombocytopen in reducing platelet adhesiveness in patients with tendency to thrombosis.

REFERENCES

1. Baar, H. S.: The stickiness of platelets. Lancet, **2**: 775, 1941.
2. Baar, H., and Székely, L.: Über die Plättchenzerfallsgeschwindigkeit bei normalen Kindern, bei der Hämophilie und Thrombopenie. Ztschr. f. Kinderh., **48**: 31–42, 1929.
3. Bernard, J., and Soulier, J. P.: Investigation of a new variety of congenital hemorrhagic thrombocytic dystrophy. Proc. International Soc. Hematology, Aug. 23, 1948, to be published.
4. Damashek, W., and Miller, E. B.: The megakaryocytes in idiopathic thrombocytopenic purpura, a form of hypersplenism. Blood, **1**: 27–51, 1946.
5. di Guglielmo, G.: Eritroleucemia e piastrinemia. Folia medica, **6**: 1–7, 36–42, 55–62, 81–88, 101–112, 1920.
6. Epstein, E., and Goedel, A.: Hämorrhagische Thrombozythämie bei vasculärer Schrumpfmilz. Virchows Arch. f. path. Anat., **292**: 233–248, 1934.
7. Giffin, H. Z., and Conner, H. M.: The untoward effects of treatment by phenylhydrazine hydrochloride. J. A. M. A., **92**: 1505–1507, 1929.
8. Harttung: Zur Verhütung der Thrombose und Embolie. Deutsche Ztschr. f. Chir., **229**: 271–276, 1930.
9. Hirschboeck, A. S., and Coffey, W. L., Jr.: Clot retraction time in thrombophlebitis and pulmonary embolism. Am. J. M. Sc., **205**: 727–730, 1943.
10. Hunter, W. C., Krygier, J. J., Kennedy, J. C., and Sneeden, V. D.: Etiology and

prevention of thrombosis of the deep leg veins; a study of 400 cases. Surgery, **17:** 178–190, 1945.
11. Ivy, A. C., Shapiro, P. F., and Melnick, P.: The bleeding tendency in jaundice. Surg., Gynec., and Obst., **60:** 781–784, 1935.
12. Kristenson; Normann; Kojima: cited by Moolten and Vroman.[14]
13. Moolten, S. E. (with the technical assistance of Hazel G. Keller and Muriel Campbell): Studies on extractable factors in the spleen and other organic sources which influence the blood platelet count. J. Mt. Sinai Hosp., **12:** 866–873, 1945.
14. Moolten, S. E., and Vroman, L.: The adhesiveness of blood platelets in thromboembolism and hemorrhagic disorders; measurement of platelet adhesiveness by the glass-wool filter. Am. J. Clin. Path., 701–709, 1949.
15. Moolten, S. E., and Vroman, L.: Unpublished studies, 1947–1948.
16. Moolten, S. E., Vroman, L., Vroman, G. M. S., and Goodman, B.: The rôle of blood platelets in thromboembolism. To be published.
17. Morrison, M.: An analysis of the blood picture in one hundred cases of malignancy. J. Lab. and Clin. Med., **17:** 1071–1093, 1932.
18. Mortensen, O.: Thrombocythemia hemorrhagica. Acta med. Scandinav., **129:** 547–559, 1948.
19. Nygaard, K. K., and Brown, G. E.: Essential thrombophilia; report of 5 cases. Arch. Int. Med., **59:** 82–106, 1937.
20. Patek, A. J., Jr., and Stetson, R. P.: Hemophilia; the abnormal coagulation of the blood and its relation to the blood platelets. J. Clin. Investigation, **15:** 531–542, 1936.
21. Quick, A. J.: Studies on the enigma of the hemostatic dysfunction of hemophilia. Am. J. M. Sc., **214:** 272–279, 1947.
22. Reid, J.: Haemorrhagic thrombocythaemia. Lancet, **2:** 584–587, 1940.
23. Rosenthal, N.: Clinical and hematologic studies on Banti's disease; the blood platelet factor with reference to splenectomy. J. A. M. A., **84:** 1887–1891, 1925.
24. Rosenthal, N.: Hemorrhagic diatheses; thrombocytopenia, thrombasthenia, capillary (hemorrhagic) toxicosis, hemophilia and thrombocythemia. H. Downey, Editor: Handbook of Hematology. New York: Paul B. Hoeber, Inc., **1:** 499–551, 1938.
25. Schürer, W.: Speicherung von Heparin in den Zellen des Reticuloendothels. Helvet. med. acta, **13:** 161–171, 1946.
26. Steiner, P. E., and Gunn, F. D.: The response of blood platelets to external stimuli: ultraviolet light, iodine and coal tar. Arch. Path., **11:** 241–254, 1931.
27. Troland, C. E., and Lee, F. C.: Thrombocytopen: a substance in the extract from the spleen of patients with idiopathic thrombocytopenic purpura that reduces the number of blood platelets. J. A. M. A., **111:** 221–226, 1938.
28. Tuchman, M. S., and Moolten, S. E.: Use of hyaluronidase in preventing pain of subcutaneous heparin injection. To be published.
29. Tuft, H. S., and Rosenfield, R. E.: Detection of intravascular clotting tendency by heparin tolerance principle; methods and clinical application. Am. J. Clin. Path., **17:** 862–865, 1947.
30. Uotila, U.: On hemorrhagic thrombocythemia. Acta med. Scandinav., **95:** 136–150, 1938.
31. von Willebrand, E. A., and Jürgens, R.: Über ein neues vererbbares Blutungsübel: Die konstitutionelle Thrombopathie. Deutsches Arch f. klin. Med., **175:** 453–483, 1933.
32. Ware, A. G., Fahey, J. L., and Seegers, W. H.: Platelet extracts, fibrin formation and interaction of purified prothrombin and thromboplastin. Am. J. Physiol., **154:** 140–147, 1948.
33. Wright, H. P.: Changes in the adhesiveness of blood platelets following parturition and surgical operations. J. Path. and Bact., **54:** 461–468, 1942.
34. Wright, H. P.: Adhesiveness of blood platelets in haemophilia. Lancet, **1:** 306–307, 1946.
35. Zucker, M. B.: Study of substances in blood serum and platelets which stimulate smooth muscle. Am. J. Physiol., **142:** 12–26, 1944. Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypoprothrombinemic rats. Ibid., **148:** 275–288, 1947.

NEURINOMA OF THE SMALL INTESTINE

Its Relative Frequency among Tumors of that Region
Report of a Case*

PAUL E. WALKER, M.D.

From the Pathology and Surgical Services, U. S. Marine Hospital, Baltimore, Maryland

A case of neurinoma of the ileum occurring at this hospital prompted a review of the subject to determine the relative frequency of this neoplasm among tumors of the small intestine.

Among approximately 50,000 hospital admissions to the Marine Hospitals in Baltimore and Norfolk from 1943 to 1947, only four diagnoses of primary neoplasm of the small intestine were made including the case of neurinoma to be reported here.

In the American literature during the last five years, over ninety articles were found on the subject of tumors of the small intestine,[6, 8, 10, 20, 23, 26] a great majority being reports of only one or 2 cases.

As a result of their study, Shallow, Eger and Carty concluded that 3 per cent of intestinal carcinomas and 60 per cent of intestinal sarcomas occur in the small bowel. However, because of the rarity of sarcoma in the intestinal tract, carcinoma is twice as frequent as sarcoma in the small bowel. Of the malignant neoplasms, lymphosarcoma ranks next to carcinoma in frequency.[6, 20] Other rarer types are leiomyosarcoma,[18] fibrosarcoma,[13] and neurosarcoma.[10, 11] Benign and malignant tumors are about of equal rarity, but the former are less often recognized clinically because, in the majority of cases, they do not produce symptoms.[6] They are most frequently found incident to surgery for some other cause or at the postmortem table.[20] In Table 1 is shown their incidence as reported in several of the larger series published since 1932.

It is to be noted that adenoma was the most frequent type seen, argentaffinoma the second commonest, and hemangioma[15] the least common. One case of osteochondroma was reported.[21] In none of the foregoing reports were we able to find a case report of neurinoma of the small intestine.

Two varieties of benign nerve sheath tumors are recognized, a differentiated and an undifferentiated form. The former, which rarely becomes malignant, occurs in myelinated nerves and is known as schwannoma, neurinoma or neurilemoma (Stout). The latter may or may not be encapsulated and is prone to recur and undergo malignant change. It occurs in nonmyelinated nerves. Varieties have been termed fibromyomas, fibroneuromas, and myxoid neuromas. They may be seen in von Recklinghausen's disease.[11, 24]

Stout in 1935,[25] in an excellent review of what he chose to call neurilemomas, was able to collect 194 adequately described cases of peripheral nerve-sheath tumors. These had been variously termed perineural fibroblastomas, schwanno-

* Received for publication, June 6, 1949.

mas, and peripheral gliomas. To these he added 52 tumors of his own, bringing the total number to 246. Forty of this total series were listed as occurring in the gastrointestinal tract. Of these, 35 were in the stomach, 2 in the first portion of the duodenum, 3 in other parts of the small intestine, 1 in the cecum, and 1 in the appendix.

To this series of 5 neurinomas of the small intestine is added one from this hospital. Fifteen adequately described cases were found in the literature (Table 2) since Stout's review. None was found in the English literature. This makes a total of 21 authentic cases of neurinoma of the small intestine.

The occurrence of these tumors was about equally divided between males and females. There were 10 males, 8 females and 3 in which the sex was not stated. Most of the tumors have been found in older persons. In 19 cases in which the age was stated, the average age was 57½ years, the youngest 29 and the oldest 73. Race was not mentioned in other case reports, but the tumor is not peculiar to

TABLE 1

RELATIVE FREQUENCY OF BENIGN TUMORS OF SMALL INTESTINE ACCORDING TO SEVERAL LARGE SERIES REPORTED SINCE 1932

	ADENOMA	ARGENTAFFINOMA	MYOMA	LIPOMA	FIBROMA	HEMANGIOMA
Rankin and Newell[21]	11		11	2	6	2
Raiford[20]	15	7	3	7	4	3
Geschickter[10]	15	10	4			4
Botsford and Seibel[6]	5	16	2	9	2	
Totals	46	33	20	18	12	9

the white race, as is shown in the case to be reported. Occurrence of this tumor with stigmata of von Recklinghausen's disease was reported in 18 per cent of Stout's general series but in none of those occurring in the intestines. Five cases in the literature[2, 7, 17, 27] had been diagnosed as schwannoma, but were omitted from this study because of inadequate pathologic description. In two of these, von Recklinghausen's disease was present.

SYMPTOMATOLOGY

The more common symptoms and signs in these 15 cases are listed in the order of their frequency in Table 3.

The onset was described as sudden in 5 cases, of four to five days' duration in one, and as insidious in 11. The average duration in those described as insidious was three years, three months. One patient had symptoms for 10 years and during that time had two isolated attacks of severe hemorrhage.[16] Two patients had no symptoms except a feeling of heaviness in the abdomen.[1, 7] It is interesting to note that pain and palpable tumor occurred in an equal number of patients and were the most common symptoms found. X-ray findings were mentioned in only one case;[2] in this case tumor or obstruction was not demonstrated.

TABLE 2

FIFTEEN CASES OF NEURINOMA OF SMALL INTESTINE LISTED SINCE STOUT'S REVIEW IN 1935

AUTHOR	AGE AND SEX	SITE	SYMPTOMS, DURATION, PRE-OPERATIVE DIAGNOSIS	SURGICAL FINDINGS AND/OR TREATMENT; RESULT	PATHOLOGIC FINDINGS
1. Serafini,[22] Italy, 1931	M 31	Small intestine	Pain, constipation, emaciation, diarrhea, palpable tumor; 7 months	Pedicled tumor wedged between bladder and rectum; excision of intestinal wall; good	Encapsulated tumor, size fetal head; varied cells; fibrillar noncollagenous tissue; round, oval nuclei; immature nerve cells
2. Borras and Benito,[5] Argentina, 1936	F 54	Ileum	Pains in lower abdomen; 45 days; ovarian cyst	Puncture of large cyst, extraction of 2 liters hemorrhagic fluid; extirpation of tumor; good	Lobulated schwannoma, size fetal head; nuclei in palisades; central degeneration, hemorrhage
3. Sjovall, cited by Hedlund,[14] Sweden, 1937	M 65	Small intestine	Constipation, anemia, palpable tumor	Resection, fist-sized tumor; recovery	Spindle cells, palisading, mitoses; schwannoma, malignant (?)
4. Sjovall, cited by Hedlund[14]	M 59	Small intestine	Palpable tumor in iliac fossa	Recovery	Necrosis; tumor, size of man's head; schwannoma
5. Arnvig, cited by Hedlund[14]	M 47	Small intestine	Pain, intestinal bleeding, anemia, palpable growth; 1½ years	Resection of goose egg-sized tumor of small intestine; recovery	Degeneration, necrosis; neurinoma
6. Bergendal and Sjovall,[4] Sweden, 1937	M 29	Jejunum	Sudden, 24 hours before operation; peritonitis from perforation	Resection 15 cm. intestine; good health 3 yr; died 3½ yr. after operation; peritoneal metastases	Fist-sized neurinoma; central cavity, communicating with intestinal lumen; perforation of tumor; spindle cells, palisading of nuclei

TABLE 2—*Continued*

AUTHOR	AGE AND SEX	SITE	SYMPTOMS, DURATION, PRE-OPERATIVE DIAGNOSIS	SURGICAL FINDINGS AND/OR TREATMENT; RESULT	PATHOLOGIC FINDINGS
7. Gaby and Gasne,[9] France, 1939	M 56	Ileum	Obstruction, palpable growth; 4 or 5 days; intestinal obstruction	Adhesion of ileum near umbilicus, resection large tumor; death on day after operation	Intestinal wall destroyed by tumor; fusiform fasciculated cells, whorls, collagen, no mitosis, no giant nuclei
8. Graffin[12] France, 1939	F 51	Small intestine	Pain; 6 months; ovarian cyst	Resection of tumor and 40 cm. small intestine; good	Orange - sized typical malignant(?) schwannoma, palisades, very large cellular atypical structures with mitoses
9. Babbini and Tettamanti,[1] Argentina, 1939	F 59	Ileum	Palpable painless mass; 5 months; mesenteric tumor	Resection tumor 50 cm. from ileocecal junction; good	Cystic schwannoma 12 cm. in diameter, fusiform cells, palisades, necrosis
10. Mouchet and Samain,[19] France, 1939	F 73	Ileum	Pain, fever, palpable mass; sudden; twisted ovarian cyst	Resection growth from ileum; no metastasis; no recidivation 15 months after operation	Encapsulated lobulated neurinoma, necrosis, communication with intestinal lumen, fusiform cells in bands, oval nuclei in palisades, collagen
11. Benniot,[3] France, 1939	M 64	Small intestine	Pain, fatigue, movable growth in hypogastrium; sudden; mesenteric cyst	Resection cyst containing 1½ liters bloody fluid; multiple soft tumors peritoneum, intestine, colon; recidivation, cachexia; death	Pedicled cyst fusiform cells, whorls, giant or vacuolar nuclei, mitosis, necrotic zones, giant or budding nuclei; schwannoma, malignant(?)

TABLE 2—*Continued*

AUTHOR	AGE AND SEX	SITE	SYMPTOMS, DURATION, PRE-OPERATIVE DIAGNOSIS	SURGICAL FINDINGS AND/OR TREATMENT; RESULT	PATHOLOGIC FINDINGS
12. Manteuffel-Szoege,[17] France, 1939	M 37	Ileum	Abdominal pain, loss of weight, palpable growth; over 8 months	Resection of tumor; results not stated	Malignant schwannoma, Antoni type A
13. Colillas and Masciottra,[7] Argentina, 1940	F 46	Jejuno-ileal junction	Painless abdominal movable mass; slow; pedicled ovarian cyst	Resection of tumor on broad pedicle; good	Schwannoma, size fetal head; palisades, microcysts, cylindromatous aspects; no nerve elements; necrosis
14. Hedlung,[14] Sweden, 1945	F 60	Ileum	Anemia, tarry stools; slow; obstructing tumor, small intestine	Resection of fist-sized tumor; good; no recurrence after 1 year	Spindle cells, sclerosis and myxomatous degeneration; palisading; central cavity connected with intestinal lumen
15. Bazterrica and Molina,[2] Argentina, 1946	F 49	Ileum	Pain, diarrhea, anemia; insidious, 5 years; mechanical chronic obstruction	Resection pedicled plum-sized tumor ileum; intestinal movements accelerated	Fusiform cells, nodules in whorls, microcystic degeneration; schwannoma

TABLE 3

SYMPTOMS AND SIGNS IN 15 CASES LISTED IN TABLE 2 IN ORDER OF FREQUENCY

Pain	13	Diarrhea	3
Palpable tumor	13	Vomiting	3
Anemia	5	Diarrhea and Constipation	2
Hemorrhage	5	Melena	2
Obstruction	4	No symptoms	2
Constipation	3	Perforation	1

DIAGNOSIS

The diagnoses made pre-operatively in this series were as follows: None, 6; ovarian cyst, 5; ileus or obstruction, 4; mesenteric tumor, 2; small intestinal tumor, 1; bleeding duodenal ulcer, 1; tumor unspecified, 1; peritonitis, 1.

An accurate pre-operative diagnosis would be unlikely in any case. However, in a person over middle age with abdominal pain and indigestion for several years, isolated hemorrhages, a palpable abdominal mass and negative x-ray findings, a presumptive diagnosis might be made. Obstruction reported in 5 cases was due to volvulus,[12] bands,[9] and in 1 case intussusception.[2] In no case was dilatation above the tumor reported at operation. Intussusception (1 case) is much rarer in this series than is reported for other benign tumors of the intestine.

TREATMENT

Resection and intestinal anastomosis was done in all cases except one and is the treatment of choice. Extirpation of the tumor without resection was possible in 1 case.[5]

PATHOLOGIC FINDINGS

Gross Features

The locations of the tumors were as follows: Two in the duodenum, 4 in the jejunum, 9 in the ileum, and 6 not stated. These tumors varied in size from 1 to 12 cm. in diameter. They were encapsulated, fusiform or egg-shaped, some were pedicled or pedunculated, and some were described as cystic. Most of them were found on the mesenteric side of the intestine but in some, their location was on the antimesenteric border. All were intimately integrated with the intestinal wall but none was described as invading the lumen. A small ulceration or a sinus tract was almost invariably present in the mucosa, and the sinus tract was occasionally described as being covered with a clot. The intestinal mucosa was strained, flattened, or atrophied but nowhere invaded. On cut-section, the tumors were well circumscribed. Cystic or necrotic degeneration was common in the larger tumors, the cavity occasionally communicating with the intestinal lumen. Small cystic areas were usually seen containing bloody fluid, while the solid portion of the tumors was white to yellowish gray or pink in color.

Microscopic Features

Two main types of tissue are described by Stout,[25] after Antoni:

Type A has the following characteristics:

1. Orderly arrangement of fibrillary or wire fibers staining dark with silver connective tissue stains.
2. Massing of fibrillary fibers in parallel array with groups of parallel nuclei at their opposite extremities, called palisading.
3. Rarely differentiation in a whorl of cells or fibers similar to that seen in meningioma.
4. Rarely no cylinder formation and only a suggestion of palisading; more rarely no palisading at all.
5. Occasionally complete necrosis with maintenance of fibrillar architecture but loss of cells.

Type B has the following characteristics:

1. No orientation of fibers or cells and lack of differentiation.

2. Loose texture of cells and fibers.
3. Microscopic degeneration.
4. Complete absence of collagen bands.

Tumors are made up in combinations of A and B types of tissue in different amounts and combinations. Vascularization is unequal and variable. The formation of dense collagen sheaths around the vessels is striking and accounts for the common degenerative changes.

In the one case of perforation reported,[4] the tumor had apparently undergone malignant degeneration and at autopsy two years later, multiple peritoneal implants were found. In a second case, recurrence occurred in a like manner two years following resection.[3]

REPORT OF CASE

A. A., a Negro woman prisoner, age 36, was admitted to the Marine Hospital, Baltimore, Maryland, on February 26, 1947. Since 1942 she had frequent attacks of weakness, dizziness and nausea. Following such attacks she had noted black, tarry stools. Occasionally she had spells of fainting and rarely vomiting. Most of her stay in prison was spent in the hospital. Her menstrual history was normal.

She was well developed and well nourished and not apparently ill. There was a soft blowing systolic murmur over the mitral area. Vaginal and abdominal examinations were normal.

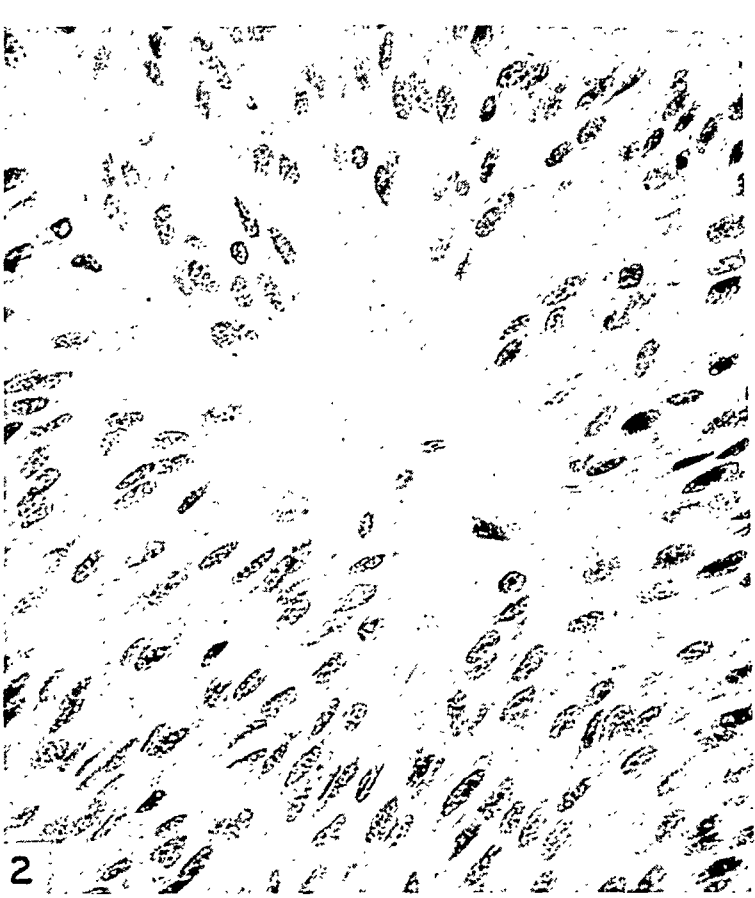
On admission, the erythrocyte count was 3,480,000, the hemoglobin 10 grams, and during hospitalization the hemoglobin fell to 6.5 grams. Sternal puncture revealed normal marrow. Bleeding and clotting times were normal. A diagnostic curettage showed normal endometrium. Examination under anesthesia showed a small pelvic mass. Occult blood in stools was reported as absent on three examinations, and markedly positive on two occasions.

On April 21, 1947, an exploratory operation was done by Dr. J. D. Lane, Chief of Surgical Service, U. S. Marine Hospital, Baltimore, Maryland. An orange-sized tumor mass was found in the mesentery of the ileum approximately 38 in. from the cecal valve. Resection and side-to-side anastomosis was done. The patient made a good recovery and was discharged from the hospital on May 10, 1947.

Grossly, the tumor was a multilocular mass measuring 8 cm. in diameter and was attached to a section of small intestine 4 cm. in length (Fig. 1). It was densely and firmly integrated with the intestinal wall, and in the area of attachment the mucosa was flattened, round, and appeared to have a minute perforation in its center. On cut section the tumor showed cystic degeneration and contained some material which seemed slightly calcific. In other areas small cysts contained bloody fluid. Several small nodular areas were present beneath the serous surface of the mass.

Microscopically, a moderately cellular tumor, appearing to have arisen in the muscular wall of the intestine, had extended to the mucosa. It consisted of fasciculated, elongated cells and fibrillar bands (Figs. 2, 3). The cells showed few short fibers lying in clear areas and often presented a loose arrangement. The walls of the blood vessels often appeared hyalinized. A fine reticular, collagenous structure was seen in various portions and was sometimes quite prominent. However, the tumor cells were in groups among these fibers but were apparently unrelated to the fibrous element. The tumor showed areas of necrosis and hemorrhage. The diagnosis of neurinoma was made by Dr. C. H. Binford, Pathologist.





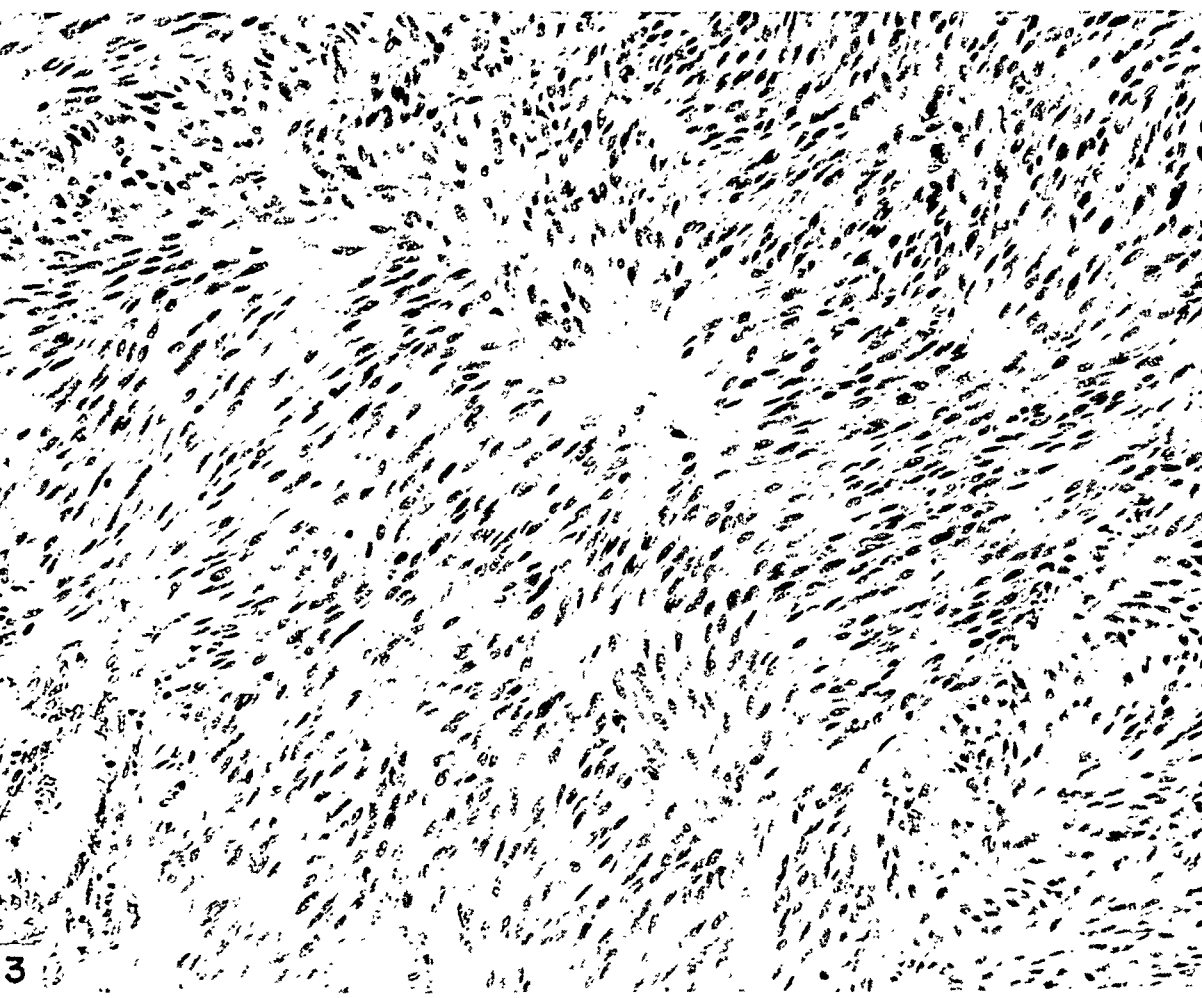


FIG. 1. Tumor of ileum at operation

FIG. 2. Photomicrograph of tumor. × 480

FIG. 3. Photomicrograph of tumor. × 200

SUMMARY

Neurinoma is a rare tumor of the small intestine. It has been found adequately described in the literature only twenty times. An additional case is reported of neurinoma which occurred in the ileum of a 36 year old Negro woman.

REFERENCES

1. Babbini, R. J., and Tettamanti, J. C.: Cystic schwannoma of small intestine; case. Bol. Soc. de cir. de Rosario, **6:** 127–131, 1939.
2. Bazterrica, E., and Vidal Molina, I.: Schwannoma of the ileum, chronic intestinal invagination. Acad. argent. de cir. Bol. y trab., **30:** 43–54, 1946.
3. Benniot, A.: Schwannoma of small intestine; case. Mém. Acad. de chir., **65:** 535–540, 1939.
4. Bergendal, S., and Sjovall, A.: Clinical and pathologic study of solitary neurinomas of the intestine. Der Chirurg., **9:** 573–579, 1937.
5. Borras, F. E., and Benito, J. V.: Schwannoma of the small intestine simulating a big ovarian cyst. Bol. Soc. obst. y ginec. (Buenos Aires), **15:** 327–339, 1936.
6. Botsford, T. W., and Seibel, R. E.: Benign and malignant tumors of the small intestine. New England J. Med., **236:** 683–694, 1947.
7. Colillas, D., and Masciottra, R. L.: Schwannoma of small intestine. Rev. med. quir. de pat. fem., **15:** 245–250, 1940.
8. Ficarra, B. J., and Marshall, S. F.: Primary carcinoma of the jejunum. S. Clin. North American, **25:** 713–718, 1945.
9. Gaby, F., and Gasne, L.: Neurinoma of small intestine. Ann. d'anat. path. (Paris), **16:** 656, 1939.
10. Geschickter, C. F.: Tumors of the digestive tract. Am. J. Cancer, **25:** 130–161, 1935.
11. Geschickter, C. F.: Tumors of peripheral nerves. Am. J. Cancer, **25:** 377–410, 1935.
12. Graffin: Solitary neurinoma of small intestine. Case. Bull. et mém. Soc. chirurgiens de Paris, **31:** 186–188, 1939.
13. Hanno, H. A., and Mensh, M.: Leiomyoma of the jejunum; intermittent melena of fourteen years duration and fatal hemorrhage. Ann. Surg., **120:** 199–206, 1944.
14. Hedlund, P.: Neurinoma of small intestine successfully removed; case. Nord. med. (Hygiea), **28:** 2343–2346, 1945.
15. Kiefer, E. D.: The small intestine; a review of recent literature. Gastroenterology, **3:** 388–398, 1945.
16. König, E.: Neurinoma of the gastrointestinal tract. Der Chirurg., **4:** 636–641, 1932.
17. Manteuffel-Szoege: Malignant schwannomas. Bull. Assoc. franç. p. l'étude du cancer, **28;** 860–870, 1939.
18. Marshall, S. F., and Welch, M. L.: Leiomyoma of the jejunum; report of case. New England J. Med., **236:** 95–97, 1947.
19. Mouchet, A., and Samain, A.: Solitary neurinoma of small intestine; case. Mém. Acad. de chir., **65:** 186–197, 1939.
20. Raiford, T. S.: Tumors of the small intestine. Arch. Surg., **25:** 321, 1932.
21. Rankin, F. W., and Newell, C. E.: Benign tumors of the small intestine; report of twenty-four cases. Surg., Gynec. and Obst., **57:** 501–507, 1933.
22. Serafini, G.: Big nervous tumor of the small intestine. Il Cancro, **2:** 281–284, 1931.
23. Shallow, T. A., Eger, S. A., and Carty, J. B.: Primary malignant diseases of the small intestine. Am. J. Surg., **69:** 372–383, 1945.
24. Stewart, F. W., and Copeland, M. M.: Neurogenic sarcoma. Am. J. Cancer, **15:** 1235–1320, 1931.
25. Stout, A. P.: The peripheral manifestations of the specific nerve sheath tumor (neurilemona). Am. J. Cancer, **24:** 751–796, 1935.
26. Weber, H. M., and Kirklin, B. R.: Roentgenologic manifestations of tumors of small intestine. Am. J. Roentgenol., **47:** 243–253, 1942.
27. Wolff, P.: Giant cystic neurinoma of small intestine with familial neurofibromatosis. Schweiz. med. Wchnschr., **17:** 379, 1936.

BRONCHIAL ADENOMA WITH CANCEROUS TRANSFORMATION*

E. F. GEEVER, M.D., W. S. WILLIAMS, M.D. AND J. E. McWILLIAMS, M.D.

From the Pathology Service of Glockner-Penrose Hospital and the Medical Service of the Union Printers' Home, Colorado Springs, Colorado

Adenoma of the bronchus is usually a benign tumor. It is not rare, approximately 300 cases having been reported up to and including Holley's study in 1946. Relatively few cases, however, have been described in which the tumor became cancerous. The purpose of this report is to present another example of maligant transformation with metastasis to the liver.

REPORT OF CASE

Clinical Data

A 74 year old white man entered the Union Printers' Hospital in April, 1948. He complained of weakness and severe shortness of breath which were present even at rest. These symptoms first appeared approximately one year previously and had become progressively severe. The patient also had a slight cough for many years which he attributed to smoking; the cough was nonproductive and its character had not changed. There was no history of hemoptysis, wheezing, chest pain or weight loss. One and one-half years prior to admission, he had suffered a "stroke" with partial paralysis in the muscles of the right side of the body. The functioning of the extremities of the right side had greatly improved in the meantime. The patient's past health had been otherwise good and there were no other significant facts in the history which were related to his present illness.

His memory for past events was poor. Ophthalmoscopic examination showed a small area of central chorio-retinitis, and narrow tortuous retinal arterioles. Percussion of the chest was hyper-resonant and the breath sounds were diminished. The blood pressure was 135/70. No other significant physical findings were noted.

Anteroposterior and lateral roentgenograms of the chest on April 5, 1948 showed a discrete, oval, tumor mass, 8.0 cm. in diameter, in the region of the right hilus. The intercostal spaces on the right appeared slightly narrower than normal (Fig. 1). X-ray examination of the skull showed no evidence of metastatic involvement. Laboratory studies revealed sputum, negative for acid-fast bacilli; urinalysis, normal; blood, erythrocytes 3,270,000; hemoglobin, 13.5 grams; leukocytes, 9350 with a normal differential count; hematocrit reading, 30 per cent; sedimentation rate, 33 mm. in 1 hour; blood nonprotein nitrogen, 41.5 mg.; Kahn, negative. On bronchoscopic examination, performed on April 13, 1948, the carina was in the midline, enlarged, and its motions were restricted; a nodular tumor mass was present in the right main bronchus near the orifice of the middle lobe; there was no significant degree of bronchial obstruction. Biopsy showed the lesion to be a bronchial adenoma.

Since both the bronchoscopic examination and the patient's clinical condition suggested the likelihood of metastatic bronchogenic carcinoma, it was elected to follow a course of palliative treatment. Roentgenogram of the chest on July 7, 1948, showed no increase in the size of the tumor mass. The clinical condition gradually became worse and the patient expired on December 1, 1948, approximately eight months after admission.

Pathologic Data

Gross findings. The right lung weighed 870 grams and showed some obliteration of the interlobar fissures by fibrous adhesions. The lung contained a discrete,

* Received for publication, May 26, 1949.









FIG. 1. X-ray of chest showing oval tumor mass in right hilar region.

FIG. 2. Cut surface of right lung. Arrow indicates site of apparent origin of tumor from bronchus of middle lobe.

FIG. 3. Photomicrograph of bronchial tumor. Cells are arranged in cords and in small gland-like formations. × 75.

FIG. 4. Metastatic lesion in liver. Cells are arranged in gland-like pattern, resembling epithelioma adenoides cysticum. × 75.

hard, tumor mass in its mid-portion on the mediastinal aspect. The tumor measured 9.0 x 7.0 cm. It was hard, and on section showed some areas that were yellow or gray and translucent and other bright yellow areas of necrosis (Fig. 2). The bronchial mucous membrane was congested and contained some blood-stained mucus. The bronchus of the middle lobe, at a point 4.0 cm. from its origin, showed infiltration by nodular yellow tissue on the side adjacent to the above-mentioned tumor mass. The dependent parts of all lobes revealed confluent lobular zones of purplish red congestion and from these areas some blood-stained fluid could be expressed. The peribronchial lymph nodes were large, measured 2.0 to 4.0 cm. in their greatest diameters and were infiltrated by yellow tissue.

The liver showed three discrete, yellowish gray, firm nodules, the largest 1.7 cm. in diameter. The cut surfaces of the nodules were also grayish yellow and identical with the tumor described in the lung.

Microscopic Findings. The right lung revealed diffuse proliferation of anaplastic epithelial cells in small and irregular glandular patterns, cords, columns and occasional sheets (Fig. 3). The cells had scanty neutrophilic cytoplasm. The nuclei were oval, indented or irregular and basophilic, and a few of them contained nucleoli. Scattered mitotic figures were apparent. In some fields the most peripheral cells were of columnar type and arranged perpendicularly to the basement membrane. Necrosis was widespread and in such areas basophilic cellular debris and occasional crystals could be identified. Argentaffin granules could not be demonstrated. Secondary infection was severe, with congestion, dense collections of polymorphonuclear cells and deposits of fibrin. The marginal pulmonary tissue also showed acute and chronic inflammatory changes and some fields with organizing fibroblasts and capillaries.

Sections of the liver through one of the metastatic tumor nodules revealed anaplastic epithelial cells in small glandular patterns, strands and cords (Fig. 4). Some nests were intravascular. The histologic characteristics were identical with those described above. Secondary necrosis and infection were marked.

DISCUSSION

Adenomas have been separated histologically into two types, carcinoid and mixed tumor. Neither designation, but particularly the latter, has been acceptable to many pathologists. Justification for the term carcinoid is weakened by the fact that the silver stains for granules are often negative. The designation, mixed tumor, has evoked greater opposition. Although the epithelial elements are similar to those in mixed tumor, other components of the latter, such as neoplastic myxochondroid elements, are often lacking. The cartilaginous tissue sometimes found is considered as a residue of the bronchial wall. Since the epithelial pattern often resembles basal cell carcinoma or its related variants, one encounters strong support for such terms as epithelioma adenoides cysticum, cylindroma or basal cell carcinoma. The subject has more than academic interest since some authors have suggested that the cylindromatous form is more likely to become malignant. Laff and Neubuerger[6] reported an example of cancerous change in the cylindromatous type. Anderson[2] also described an example of cancer which had

histologic features similar to cylindroma. Holley[4] reported 38 tumors, of which three were malignant and examples of the carcinoid type and two of the mixed tumor variety. Others have reported cancerous forms or adenomas of doubtful malignancy without attempting to clasify the lesions histologically. Moersch, Tinney and McDonald[7] reported one example of cancerous transformation in their series of 38 bronchial adenomas. Clerf[3] found only one malignant form in a series of 55. Adams, Steiner and Bloch[1] reported 5 cases of bronchial adenoma, three with cancerous transformation. Jackson and Konzelmann,[5] in a series of 12 tumors, reported one case in which the "ultimate outcome is doubtful". From the histologic examination of their photomicrographs, this tumor also resembled epithelioma adenoides cysticum.

Recently Small and Hankins[8] reported 2 cases of basal cell carcinoma of the skin with metastasis to the lungs in addition to other organs. In one case the pulmonary metastases encroached upon or occluded the main bronchus of the upper lobe. This report emphasizes the need for careful, complete physical examination and/or necropsy before attributing local origin to bronchial tumors of this type.

Some consideration might be given to the possibility that this tumor was cancerous from the onset of its evolution. The history is not helpful. The duration of symptoms over a period of 20 months could fit in with either a benign or cancerous lesion. The large size of the primary focus, the presence of metastasis to the liver and the microscopic appearance of anaplasia suggest that cancer was present from the very beginning. However, the histologic features do not fit any known pattern of primary bronchogenic carcinoma. On the other hand, cancerous changes in bronchial adenomas with histologic characteristics resembling basal cell carcinoma or its variants have been described previously. Death in this case was due to complicating acute and chronic organizing pneumonia. Widespread metastases, so often present in bronchogenic carcinoma, were not discovered. It is our belief that this was a bronchial adenoma which underwent cancerous change.

SUMMARY

1. A case is presented of bronchial adenoma with cancerous change and metastasis to the regional lymph nodes and liver in a 74 year old white man.

2. The histologic characteristics were similar to those encountered in epithelioma adenoides cysticum of the skin.

REFERENCES

1. Adams, W. E., Steiner, P. E., and Bloch, R. G.: Malignant adenoma of the lungs; carcinoma-like tumors with long clinical course. Surgery, **11**: 503–526, 1942.
2. Anderson, W. M.: Bronchial adenoma with metastasis to the liver. J. Thoracic Surg., **12**: 251–360, 1943.
3. Clerf, L. H.: Adenoma of bronchus. Ann. Otol. Rhin., and Laryng., **57**: 869–874, 1948.
4. Holley, S. W.: Bronchial adenomas. Mil. Surgeon, **99**: 528–554, 1946.
5. Jackson, C. L., and Konzelmann, F. W.: Bronchoscopic aspects of bronchial tumors, with special reference to so-called bronchial adenoma. J. Thoracic Surg., **6**: 312–329, 1937.
6. Laff, H. I., and Neubuerger, K. T.: Bronchial adenoma with metastasis. Arch. Otolaryng., **40**: 487–493, 1944.
7. Moersch, H. J., Tinney, W. S., and McDonald, J. R.: Adenoma of the bronchus. Surg., Gynec. and Obst., **81**: 551–558, 1945.
8. Small, C. S., and Hankins, T. D.: Basal cell carcinoma with metastasis. Arch. Path., **47**: 196–204, 1949.

PARACOLON ENDOCARDITIS*

IRVING A. FRIEDMAN, M.D., AND MILTON GOLDIN, M.S.

From the Departments of Medicine (Service of Dr. Earl Zaus), Bacteriology and Pathology, and the Hektoen Institute for Medical Research of Cook County Hospital, Chicago, Illinois

Endocarditis caused by organisms of the family Enterobacteriaceae is rarely found. *Escherichia coli* endocarditis has been encountered infrequently.[5, 7, 11] Fletcher,[5] in 1947, reviewed 11 authentic cases and added one of his own. *Aerobacter aerogenes, Klebsiella pneumoniae, Salmonella typhosa, Salmonella paratyphi B*,[17] and other members of this family have also been listed as the causative agents, but are even less often diagnosed.

To our knowledge, the only previous case of paracolon endocarditis reported, has been that of Robertson;[12] it involved the pulmonary valve, was secondary to infected polycystic kidneys, and occurred in a 40 year old white man.

We believe that the present case is of significance and interest, not only because of the extreme rarity of the occurrence of the infecting organism in endocarditis, but also because of the therapeutic problems involved.

REPORT OF CASE

The patient, a 54 year old white woman, first entered Cook County Hospital on July 17, 1948, with a history of diabetes which had not been treated with insulin. She had marked polyuria, polydypsia, and dysuria since March 1948, and had lost about 21 pounds. She complained of abdominal pain lasting four days, with associated nausea and vomiting, followed by fever, chills, and lethargy. On physical examination she was well developed, well nourished, but anemic and lethargic. The blood pressure was 136/86, pulse rate 88 per minute; and temperature 97 F. The left ventricle was enlarged but no murmurs were heard. Moist rales were present at the base of the right lung posteriorly. The liver was barely palpable and there was right costovertebral tenderness.

Laboratory examinations revealed 67 per cent hemoglobin, 3.4 million erythrocytes, 7500 leukocytes, with 94 per cent polymorphonuclears and 6 per cent lymphocytes, urine albumin 2 plus, sugar 4 plus, acetone trace, sediment negative; blood sugar on admission 430 mg. per 100 ml. of blood; CO_2-combining power 42 per cent, nonprotein nitrogen 53 mg.; total proteins 5.8 Gm., albumin 3.8 Gm. and globulin 2.0 Gm.; Kahn test negative.

The patient developed a fever of 101 F. soon after admission. X-ray film of the chest revealed an increase in the transverse diameter of the heart and an elevation of the right diaphragm. A flat plate of the abdomen showed some enlargement of the left kidney. Urine culture on July 19 revealed the presence of a paracolon organism. No blood culture was taken at that time. The patient had mild diabetic acidosis and infection and was treated with insulin, fluids administered orally and intravenously, sulfadiazine and penicillin. After three or four days the fever subsided, the diabetes was brought under control, and the patient was discharged to the outpatient department.

The patient again entered the hospital on August 4, 1948, because of nausea, vomiting, chills, fever and weakness of four days' duration. Physical examination at this time revealed her to be acutely ill, febrile and anemic. Blood pressure was 150/95, pulse rate 104 per minute and temperature 105 F. Left ventricular enlargement was still present, but in addition there was also a loud systolic murmur over the mitral area, transmitted to the axilla. Examination also revealed moist rales in the base of the right lung posteriorly, a barely

* Received for publication, June 28, 1949.

palpable liver and a nonpalpable spleen, costovertebral tenderness on the right side, and no petechiae. A blood count revealed 55 per cent hemoglobin, 3.02 million erythrocytes, 7600 leukocytes, 91 per cent polymorphonuclears, 9 per cent lymphocytes; sedimentation rate 26 mm. in one hour (Wintrobe, corrected); urinalysis 1 plus albumin, no sugar or acetone, and negative microscopic examination; nonprotein nitrogen from 26 to 40 mg.; blood sugar 290 mg. on admission, but 100 mg. after diabetic control; CO_2-combining power 46 per cent; total protein 5.1 Gm. with 2.7 Gm. albumin and 2.4 Gm. globulin; serum chlorides 92.3 mEq./liter and serum sodium 129 mEq./liter; agglutination tests with typhoid "H" and "O", paratyphoid A and *Brucella abortus* antigens negative; agglutinins present with paratyphoid B antigen, in 1:160 titer.

Soon after admission the patient experienced severe chills, and within 24 hours developed marked meningismus, but no Brudzinski's or Kernig's sign was elicited. A spinal tap revealed a pressure of 250 mm. of water, with clear fluid which contained a rare white cell. The Pandy and Wassermann reactions were negative, protein content was 40 mg., glucose 85 mg., and a culture of the cerebrospinal fluid showed nonhemolytic *Staphylococcus albus*, which was considered a contaminant. Several electrocardiograms showed only low T waves in leads 1 and 3. An x-ray film of the chest revealed an enlarged left ventricle with increased density in the right lower lung field. An intravenous pyelogram suggested hydronephrosis on the right side with tortuosity and dilatation of the right ureter. Blood cultures yielded paracolon organisms on August 5, 6, 10, 16 and 21. Cultures of the stool were positive for the same organism on August 8, 9, 10, and of the urine on August 6 and 10.

The patient continued to have fever. Changes were noted daily in the intensity of the mitral murmur which, at times, became whistling and had a marked diastolic component. In view of the laboratory and physical findings, it was believed that this patient had a severe paracolon bacteremia and endocarditis, originating from an infection of the kidneys. *In vitro* sensitivity tests of this bacillus showed the organism to be inhibited by 12.5 micrograms of streptomycin and 83.3 Oxford units of penicillin. It was recognized that the organism was resistant to both of these antibiotics and the most vigorous treatment practicable was attempted. The patient was placed on streptomycin, 2 Gm., 1.5 million units of penicillin, 5 Gm. of intravenous sulfathiozole each day and was repeatedly given blood transfusions. Ten days after admission the temperature was still 103 F., the leukocyte count rose to 15,000 and the patient appeared "toxic" and weak. Surprisingly, her diabetes remained well controlled on very small doses of protamine zinc insulin. On August 21, 1948, the patient had two generalized convulsions, each lasting several minutes; her respirations became labored and she required oxygen; the neck became rigid and the right upper extremity flaccid; and on this day she expired.

PATHOLOGIC REPORT

Autopsy was performed two hours after death by Dr. Bruno W. Volk. About 500 cc. of serosanguinous fluid was present in each thoracic cavity. The posterior aspect of the lower lobes of both lungs were atelectatic and all lobes were congested.

The heart weighed 465 grams. The epicardium was smooth and shiny, and the myocardium was beefy red and showed small gray-white scars in the posterior wall of the left ventricle. The wall of the left ventricle was 2 cm. thick, and that of the right ventricle 4 mm. The endocardium was smooth and thin and the papillary muscles and trabeculae were hypertrophic. At the anterior leaflet of the mitral valve there was a large firm vegetation, 1.5 x 1.5 x 1 cm., attached to the endocardium. On sectioning, it was composed of a purplish, lamellated tissue. The chordae tendineae were thin and delicate. The mitral ring measured





FIGS. 1 and 2

9 cm., the tricuspid ring 11.5 cm., the aortic ring 7.5 cm., and the pulmonic ring 8 cm. The coronary arteries were patent and their intima as well as that of the aorta were studded with a considerable number of atheromatous plaques. Microscopically, there was noted scanty infiltration of the interstitial connective tissue of the myocardium consisting of round cells and few polymorphonuclear cells. The ring of the mitral valve revealed fibrosis with hyalinization. The base of the valve was infiltrated with segmented and round leukocytes, and there was an occasional capillary. Deposits of fibrin surrounding large colonies of bacteria were seen in the vegetation of the mitral valve. Newly-formed capillaries surrounded by fibroblasts and segmented leukocytes grew into the vegetation. Postmortem culture of blood was positive for paracolon organisms.

The spleen weighed 410 grams. Its capsule was slate-gray in color and smooth. The cut surface scraped with ease and showed several small anemic infarcts. Histologically, the lymph follicles were large and hyperplastic, and the pulp showed marked proliferation of the reticulum cells.

In the right adrenal gland was a hazelnut-sized walled-off abscess which was filled with creamy yellow pus and necrotic material. This abscess involved the cortex and medulla. Smears of this pus showed large numbers of gram-negative bacilli.

Each kidney weighed 230 grams. Their capsules stripped with ease, leaving a smooth pink surface which showed some yellow mottling and occasional pinpoint hemorrhages. In addition, several saddle-shaped scars were noted. In the left kidney a recent anemic infarct, 1.5 x 1.5 cm., was found. The pelvis was lined by pale pink, smooth mucosa. Microscopically the glomeruli were large and cellular with proliferation of endothelial cells and infiltration with segmented leukocytes. The small arteries and arterioles displayed moderate thickening and hyalinization of the wall, especially of the intima. Occasionally one of the larger arcuate arteries showed a small mural thrombus. The renal pelvic mucosa was edematous and showed scattered infiltration with round cells.

The ureters were patent. The bladder, which contained cloudy yellow urine, displayed an edematous and congested mucosa with occasional small submucosal hemorrhages.

In summary, the autopsy revealed subacute bacterial endocarditis with some myocardial fibrosis; left ventricular hypertrophy; pulmonary congestion and edema with bilateral hydrothorax; reactive hyperplasia of spleen with small anemic infarcts; cloudy swelling of liver; acute glomerulonephritis with anemic infarct of left kidney; bilateral chronic pyelonephritis; chronic moderate cystitis; abscess of right adrenal; cerebral edema.

DISCUSSION

The significant bacteriologic findings were as follows: During the first admission, paracolon bacilli were isolated from urine. During the second admission, blood cultures were positive for paracolon organisms on seven successive occasions; blood drawn just before death was sterile; the same organisms were found in the stool three times and in the urine twice; at post mortem, the blood culture revealed the same organism, the spleen was sterile, smears from the adrenal abscess showed numerous gram-negative bacilli, but unfortunately, no specimen from this abscess was taken for culture.

In every case, the organism isolated was a motile, gram-negative, short, plump rod. Colonies on E.M.B. and S.S. agar measured 2 to 4 mm. in diameter, ap-

FIG. 1. Photograph of large firm vegetation, anterior leaflet of mitral valve.

FIG. 2. Photomicrograph from section of vegetation showing colonies of bacteria surrounded by fibrin. × 50.

peared smooth, translucent and slightly grayish, was not hemolytic on blood agar, its "IMViC" formulation was ++−−; it fermented dextrose, trehalose, salicin and maltose with gas formation within 24 hours; it fermented sucrose and lactose only after 14 days' incubation; it did not ferment cellobiose and did not liquefy gelatin during the 21-day period of observation. Biochemically, this organism appears to be closely related to type 28221 of Stuart *et al.*[16] and would be classified, according to Bergey,[2] as *Paracolobactrum coliforme*. This strain, however, was agglutinated by antiserum to type P29911 of Stuart, Wheeler and McGann[15] and is thus closely related serologically to this anaerogenic organism, which in turn is probably identical with the "Wakefield" type of dysentery organism described by Berger.[1]

Paracolon bacilli appear to be intermediate between coliform organisms on the one hand and the salmonellae and shigellae on the other. Serologic and biochemical reactions are not constant enough to permit definitive species classification. The pathogenicity of this group for human beings has been repeatedly investigated and reported.[3, 4, 9, 10, 14, 16] Invariably, however, these organisms are traced to gastrointestinal or genitourinary tract infections. Cases of proven pathogenicity in other situations are extremely rare. In this case, the repeated isolation of the organism in pure culture from various sources, both antemortem and postmortem, and the clinical picture, seemed to make it unequivocal that this organism was actually the responsible etiologic agent. The pathogenicity of an organism of reputedly low invasive power may, in such a case, be related to the patient's debility, diabetes, anemia and lack of demonstrable immunologic response. The patient's serum agglutinated a commercial antigen of *S. paratyphi B* to a titer of 1:160, but did not agglutinate the homologous organism on repeated attempts. It is likely that this organism shared some antigens in common with *S. paratyphi B*. Paracolon organisms with antigens of other members of the family Enterobacteriaceae have been reported many times.[4, 6, 13, 16]

In this case, it is believed that the probable sequence of events was first a chronic pyelonephritis in a diabetic, then a bacteremia with localization on the valves of an arteriosclerotic heart. There was no demonstrable evidence of a rheumatic or congenital lesion. On her first admission the patient presented evidence of mild arteriosclerotic heart disease and no murmurs were heard. The occurrence of a harsh systolic murmur at the time of her second admission 18 days later, without marked change in her hematologic picture, plus the laboratory findings constituted strong evidence for endocarditis rather than anemia as the cause of the murmur.

The cystitis and ascending pyelonephritis was shown at autopsy to be of a chronic nature. Other sequelae of the bacteremia included a septic spleen and abscess formation of the right adrenal. Once the diagnosis was established, the treatment of this patient presented a difficult problem. Attempts were made, of course, to increase the general resistance of the patient and to combat the anemia with blood transfusions. The diabetes was easily controlled but the organism was insensitive *in vitro* and apparently *in vivo* to penicillin and streptomycin, and sulfathiazole was ineffective.

CONCLUSIONS

A case of subacute bacterial endocarditis and bacteremia caused by *Paracolobactrum coliforme* is reported. The clinical, pathologic and bacteriologic data, the probable sequence in the development of the endocarditis, and the therapeutic difficulties encountered are presented.

REFERENCES

1. BERGER, F. M.: A new non-mannite fermenting dysentery organism of the Flexner group. J. Hyg., **44**: 116–119, 1945.
2. BREED, R., Ed.: Bergey's Manual of Determinative Bacteriology. Ed. 6. Baltimore: The Williams and Wilkins Company, 1948.
3. EDWARDS, P. R., WEST, M. G., AND BRUNER, D. W.: Arizona group of paracolon bacteria. Kentucky Agric. Exper. Sta. Bull. No. 499, 1947.
4. FELSENFELD, O., AND YOUNG, V. M.: The importance of paracolon organisms with Salmonella and Shigella antigens. Am. J. Digest. Dis., **12**: 396–398, 1945.
5. FLETCHER, D. E.: *Bacillus coli* endocarditis; review of the literature with report of a case. Am. Heart J., **34**: 743–750, 1947.
6. GARD, S., AND ERICKSON, E. J.: Studien ueber Colistaemme mit Salmonella-H antigenen. Ztschr. f. Hyg. u. Infectionskr., **122**: 54–61, 1939.
7. HARRIES, G. E., AND BURTENSHAW, J. M. L.: Acute infective endocarditis due to *Bacterium coli*. Lancet, **233**: 803–804, 1937.
8. HORDER, T. J.: Infective endocarditis with an analysis of 150 cases and with special reference to the chronic form of the disease. Quart. J. Med., **2**: 289–324, 1908.
9. LUIPPOLD, G. F.: Paracolon organism antigenically related to the Sachs Q-1030 bacillus and associated with chronic enterocolitis. Gastroenterology, **8**: 358–366, 1947.
10. NETER, E., AND CLARK, P.: Observations on the possible pathogenicity of paracolon bacilli as incitants of diarrheal diseases in infants and children. Am. J. Digest. Dis., **11**: 356–360, 1944.
11. PHIPPS, C.: Acute bacterial endocarditis. New England J. Med., **207**: 768–770, 1932.
12. ROBERTSON, T.: Paracolon bacillus endocarditis of the pulmonic valve secondary to infected polycystic kidneys. Arch. Path., **43**: 318–323, 1947.
13. SAPHRA, I., AND WASSERMAN, M.: Serological relationships amongst Salmonella and other *Enterobacteriaceae*. J. Immunol., **50**: 221–227, 1945.
14. SHAUB, I. G.: Paracolon bacilli and their relationship to urinary tract infections. J. Lab. and Clin. Med., **31**: 958–973, 1946.
15. STUART, C. A., WHEELER, K. M., AND MCGANN, V.: Further studies on one anaerogenic paracolon, type 29911. J. Bact., **52**: 431–438, 1946.
16. STUART, C. A., WHEELER, K. M., RUSTIGAN, R., AND ZIMMERMAN, A.: Biochemical and antigenic relationships of the paracolon bacteria. J. Bact., **45**: 101–119, 1943.
17. WELLS, H. G.: Acute endocarditis produced by *Bacillus paratyphosus B*. Arch. Path., **23**: 270–274, 1937.

DILATATION OF THE ACINI OF THE PANCREAS IN HYPEREMESIS OF PREGNANCY*

PH. H. HARTZ, M.D.

From the Public Health Service, Curaçao, N.W.I.

Recent publications have shown that lesions similar to those present in the early stages of fibrocystic disease of the pancreas in children frequently occur in adults. Baggenstoss[1] reported dilatation of the acini of the pancreas with flattening of the epithelial cells and inspissation of secretion in 121 (45 per cent) of 270 cases of uremia. In a control series of 200 cases the lesion was present in 20 per cent; in 11 of these 40 cases there was intestinal obstruction.[2] Hartz[4] observed in 6 out of 171 autopsies, in which the pancreas was examined one hour or less after death, sometimes only a few minutes after death, an increased firmness of this organ without other gross changes. On microscopic examination of this "firm pancreas" lesions similar to those described by Baggenstoss[1, 2] were observed but in addition cyst formation in the islets and sometimes mitoses in the acinar cells and in the epithelium of the intercalated ducts and of the islets. Menten and Kinsey[6] described asymptomatic retention of pancreatic secretion in 35 of 256 autopsies on children. The ages of their patients ranged from 10 days to 16 years. Hartz[5] reported the occurrence of a "firm pancreas" with typical histologic findings in 3 children whose ages were 7, 19 and 24 months. In the cases reported by Menten and Kinsey[4] and Hartz[5] there was no fibrocystic disease.

According to Baggenstoss,[3] the following factors are important in the pathogenesis of the lesions in adult persons: (1) Inhibition of the type of pancreatic secretion, normally stimulated by secretin, which may be caused by vomiting, by distention of the stomach or by mental discomfort; (2) nervous stimulation of the pancreas which leads to the formation of a juice rich in protein; and (3) dehydration, causing inspissation of the juice.

Finally malnutrition, especially protein deficiency, may result in failure of reparative protein synthesis in the acinar cells. As the pancreas in its secretion puts out 5 or 6 Gm. of protein a day,[7] protein deficiency may play an important part. Since vomiting, dehydration, malnutrition and even mental discomfort are typical symptoms of hyperemesis of pregnancy, the occurrence of dilatation of the pancreatic acini is to be expected in this disease, and it was deemed of interest to examine the pancreas in fatal cases of hyperemesis coming to autopsy.

With modern managment, a fatal outcome in hyperemesis of pregnancy is very rare; nevertheless, I have observed 3 such cases among 2100 consecutive autopsies performed at Curaçao. In 2 of these cases pancreatic tissue was available for microscopic study.

* Received for publication, June 1, 1949.

REPORT OF CASES

Case 1

A Negro woman, 30 years old and in the fourth month of pregnancy, entered the hospital in a critical condition and died approximately 12 hours after admission. The clinical diagnosis was hyperemesis of pregnancy. Autopsy was performed five minutes after death. The gross findings except for emaciation and some fatty infiltration of the liver were essentially negative.

Microscopic findings. Tissues were fixed in Heidenhain's Susa mixture and embedded in paraffin. The sections were stained with Masson's trichrome, with the azan method, with Petersen's acid alizarin blue-aniline blue and with the periodic acid-leucofuchsin method. In every lobule of the pancreas, varying numbers of dilated acini and terminal intercalated ducts were observed. They were quite similar to those described in previous publications. Also in many lobules there was moderate atrophy of the nondilated acini and vacuolization of acinar cells. A few mitoses were found in acinar cells and in the epithelium of the intercalated ducts. The majority of the islets were normal; they were sometimes quite large; the alpha cells stained well with the azan and trichrome stains. Only an isolated islet showed cyst formation. The larger ducts were normal; there was no squamous metaplasia of the epithelium. In the interlobular connective tissue scattered lymphocytes were found. Irregularly distributed through the lobules of the *liver* were dark-staining liver cells which often contained pyknotic nuclei. In the centers of the lobules the liver cells were vacuolated and contained many strongly Schiff-positive granules, probably glycogen. The *kidneys*, *suprarenal glands* and *spleen* showed nothing abnormal. In the *uterus* there was pronounced chorionic invasion of the myometrium.

Case 2

A Negro woman, 45 years old and in the fourth month of pregnancy, entered the hospital in critical condition and died after 24 hours. Autopsy was performed 10 minutes after death. A few subcapsular hemorrhages and some fatty infiltration were observed in the liver. In addition there was slight bilateral hydronephrosis and shallow ulceration of the mucosa of the terminal part of the esophagus.

Microscopic findings. The methods used were the same as in Case 1. Dilatation of the acini of the *pancreas* was present in every lobule but in a lesser degree than in Case 1. Vacuolization and atrophy of acinar cells were often combined with pronounced pyknosis of the nuclei. In addition there were frequently areas of swelling of so-called centro-acinar cells with atrophy of the acinar cells. The islets were normal; after examining many slides one islet with cyst formation was found. The interlobular connective tissue and the larger ducts showed nothing abnormal. In the *liver* there was moderate central fatty infiltration. The esophagus showed superficial ulceration with subacute inflammation, which sometimes extended into the muscularis. The other organs showed no pathologic changes.

COMMENT

Examination of the pancreas in these cases showed dilatation of the acini, as has been postulated. In contrast to the cases reported previously,[3] cyst formation in the islets was very rare and only in Case 1, in which the lesion was more advanced, could a few mitoses be observed. In Case 2 the dilatation of the acini was moderate. This may be due to different factors, which cannot be definitely evaluated because of lack of sufficient clinical data. Differences in duration of the illness or in the intensity of nervous stimulation may have played a role.

In uremia, congestive heart failure, intestinal obstruction and in ulcerative

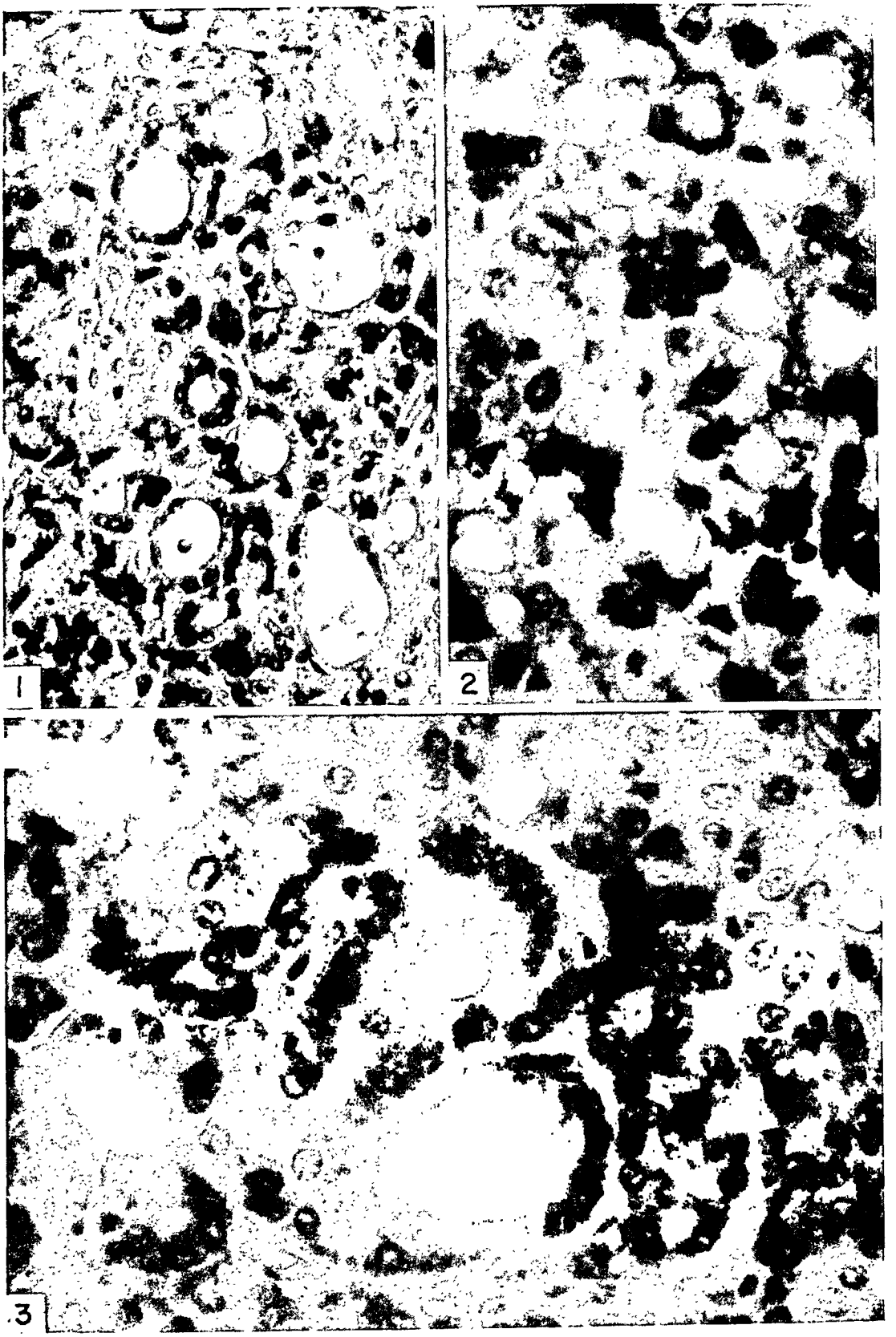


FIG. 1. Case 1. Dilatation of acini. × 230 FIG. 2. Case 2. Vacuolization of acinar cells. × 500
FIG. 3. Case 2. Dilatation of acini and swelling of epithelium of intercalated ducts. × 500

colitis, diseases in which dilatation of the acini is frequent, many factors which may cause change in the pancreas other than dilatation of the acini may be operative for a long period. In the present cases of hyperemesis of pregnancy, as shown by the result of the autopsy no such complicating factors were present, so that the changes found in the pancreas must be ascribed solely to the vomiting, malnutrition and dehydration. They offer, therefore, strong evidence for the concept of Baggenstoss[6] regarding the etiology of the dilatation of the acini of the pancreas and show once more that lesions of the pancreas in various diseases are more common than is generally assumed.

SUMMARY

In two fatal cases of hyperemesis of pregnancy dilatation of the acini of the pancreas was found. The importance of these findings for the concept of the etiology of similar lesions of the pancreas is briefly discussed.

REFERENCES

1. Baggenstoss, A.: The pancreas in uremia; a histopathologic study. Am. J. Path., 24: 1003–1017, 1948.
2. Baggenstoss, A.: Dilatation of the acini of the pancreas; incidence in various pathological states. Arch. Path., 45: 463–473, 1948.
3. Baggenstoss, A., Power, M. H., and Grindlay, J. H.: The relation of fibrocystic disease of the pancreas to a deficiency of secretin. Pediatria, 1: 435–442, 1948.
4. Hartz, P. H.: Over het vaste pancreas. Nederl. tijdschr. v. geneesk., 92: 1217–1222, 1948.
5. Hartz, P. H.: Het voorkomen van het vaste pancreas bij kinderen. Nederl. tijdschr. v. geneesk., 93: 1517–1522, 1949.
6. Menten, M. L., and Kinsey, W. C.: Asymptomatic retention of pancreatic secretion. Arch. Path., 47: 90–96, 1949.
7. Waterlow, J. C.: Discussion of *Malignant Malnutrition* (Kwashiorkor) by H. C. Trowell. Tr. Roy. Soc. Trop. Med. and Hyg., 42: 434–435, 1949.

BOOK REVIEWS

Krebsmetastasen. By DR. MED. HANS E. WALTHER, Medical Director of the X-ray Division of the Nurses Home of the Red Cross, Zurich. 560 pp., 293 illustrations. 60 francs ($14.40). Basel: Benno Schwabe and Company, 1948.

This extensive monograph deals not only with the localization of metastases of the various tumors, but considers the various routes and mechanisms of metastases, and the fate of tumor emboli in the absence of metastases. It also deals with contact metastases, accidental transplantation by means of the surgical knife. Mention is also made of tumor transplanted from one patient to another, which possibility, however, is considered to be mythical. The author, in quoting Borst, makes use of the terminology "premetastatic phase" which he defines as the interval between the time of occurrence of emboli in the circulating blood and the localization of tumor emboli with secondary tumor growth.

Various factors are discussed which may or may not influence the production of metastases, such as, infectious diseases, therapeutic measures (principally removal of tissue for biopsy) and the result of pertinent experimental work. He again confirms the present-day impression that excision of tissue for biopsy properly done, causes much less damage than repeated clinical palpation of the tumor.

The second part of the monograph deals with the localization of metastases from primary tumors to the various organs or system of organs. This part is particularly valuable because with the exception of individual reports of isolated instances of carcinoma, only little can be found in the older or in the recent literature on this subject. It is interesting that the author comments on certain discrepancies in the literature in regard to the frequency of metastases in various organs. In discussing primary carcinomas of the breast, he remarks that some authors, in a series of cases, found metastases in the suprarenals in only three instances, whereas another investigator encountered metastases 32 times. Such differences, he believes, are difficult to explain. He assumes that this discrepancy may be explained on the basis that some investigators counted metastasis to both suprarenals as two. However, it seems much more likely that the one author who found metastases in 32 instances relied on microscopic investigations, and examination of numerous sections, whereas the investigator who found only a few metastases either relied on gross examination or cut too few sections. It is the experience of the reviewer that in certain instances of breast carcinoma, a number of sections have to be cut before a particular organ can be declared free from metastases.

It is stated that this book has not been written by a pathologist, but by a roentgenologist. Yet, it gives very good gross and microscopic accounts of the various tumors discussed. There are a number of excellent illustrations included and many valuable tables. The printing and the paper are excellent. The book is written in German, which is usually concise and clear, avoiding complicated phraseology.

In short, it is a very good monograph which fills an important need for the physician in general practice, who should be aware of the possible localizations of metastases in a patient with carcinoma, and also for the investigator who should welcome a handy reference book.

Chicago OTTO SAPHIR

Microbiology and Man. ED. 2. By JORGEN BIRKELAND, PH.D., Professor of Bacteriology, Ohio State University, Columbus, Ohio. 525 pp., 54 figs., 14 tables. $5.00. Baltimore: The Williams & Wilkins Company, 1949.

In the preface to the first edition, the author states: "This book is designed as an elementary text for the student who plans to take but one or two courses in microbiology. It is intended to serve as a basis for an understanding of the part played by microorganisms in everyday life." A reprinting two years after initial publication followed by a second reprinting eight months later would seem to indicate that this book has met a definite need. Revision in the second edition tends to bring both general and specific information more

nearly up to date. The text now includes basic information in reference to electron-microscopy, antibiotics, the more recent rickettsial and virus diseases and recent advances in bacteriologic technics.

The over-all format conforms closely to that of other general texts in microbiology. Fundamentals of Bacteriology comprises the first section of 135 pages. Forty pages are devoted to infection and resistance. Common infectious diseases are discussed in section 3 (210 pages), the general plan of the discussion of each disease following the same pattern of symptomology, etiology, epidemiology, prophylaxis and treatment. The final section is devoted to microbiology of air, food, milk, water, sewage and soil. The glossary at the end of the text is a useful addition to the book.

The first two and the final sections of this text book fulfill the author's stated objective quite adequately. Those portions of the sections on infectious disease which deal with bacteriology, immunology and epidemiology are well written and informative. One may wish, however, that the discussion of bacillary dysentery had included some recognition of current serologic methods for identification and classification of these organisms. The author does not seem to be on as firm ground when he attempts to discuss the clinical classification of disease or their treatment. One can hardly agree, for instance, with the author's description of the childhood type of tuberculosis as miliary tuberculosis. One might also question his statement that syphilis is the most common cause for miscarriage or abortion. One may also doubt if the clinical bacteriologist would agree that it is safe to infer that gram-negative oxydase-positive diplococci isolated from urogenital cultures are probably gonococci The discussion of the treatment of tuberculosis includes no mention of streptomycin therapy aside from its use in tuberculous meningitis. There is no mention of serum therapy in *Hemophilus influenzae* infection.

Lansing, Michigan H. E. Cope

Cardiac Catheterization in Congenital Heart Disease, A Clinical and Physiological Study in Infants and Children. By André Cournand, M.D., Associate Professor, Department of Medicine, College of Physicians and Surgeons, Columbia University, Janet S. Baldwin, M.D., Assistant Professor, Department of Pediatrics, New York University College of Medicine and Aaron Himmelstein, M.D., Instructor, Department of Surgery, College of Physicians and Surgeons, Columbia University. 108 pp., illustrated. $4.00. New York: The Commonwealth Fund, 1949.

Dr. Cournand and his associates have published a timely monograph on the application of cardiac catheterization to the study of congenital heart disease in children. It is apparent that such studies become more and more important as surgical procedures develop.

Part one deals with the physiologic methods and technic. The information presented is concise and yet sufficiently detailed for innovation of cardiac catheterization in any well-equipped and well-staffed hospital. Formulae for blood flow through various chambers and vessels are presented in readily understandable mathematical terms. Some difficulty may occur in the application of the formulae to specific cases because of the lack of explanation for their derivation.

The second part includes studies made on extremely interesting and unusual cases of congenital heart disease exemplifying the complexity and the theoretical, as well as the practical, problems involved in congenital heart diseases. Not all cases seem to show sufficient differences in blood oxygen concentrations in the various chambers and vessels for differential diagnosis but other correlative observations, such as pressure differences and passage of catheter into various compartments via abnormal route, make the final diagnosis of each case quite convincing. Important clinical and physiologic relationships are presented. Unique diagrammatic representation of physiologic data affords the reader immediate appreciation of the degree and nature of the functional abnormality once he or she becomes accustomed to the symbols used in the diagrams.

Detroit L. T. Iseri

An Atlas of Bone-Marrow Pathology. By M. C. G. ISRAËLS, M.Sc., M.D., M.R.C.P., Lecturer and Deputy Director, Department of Haematology, The University and Royal Infirmary, Manchester; Late Foulerton Research Fellow of The Royal Society; Wing-Commander, R.A.F.V.R. 52 pp., 12 plates, 6 tables. $6.50. New York: Grune and Stratton, 1948.

There is very little which this reviewer can find in this small volume to recommend it. The presentation of descriptions of the various blood cells is over-simplified for anyone who has been taught the elements of hematology in medical school. The colored plates have been made from drawings, rather than photomicrographs, and the cells often appear pale and poorly stained. For the trained hematologist or clinical pathologist the book is of little value. The practicing physician who is interested in "cells" will do much better with the recent texts in hematology which have better plates and descriptions.

Brooklyn, N. Y. LEO M. MEYER

Fundamentals of Pulmonary Tuberculosis and Its Complications. Sponsored by the American College of Chest Physicians. Editor, EDWARD W. HAYES, M.D.; Editorial Committee, ANDREW L. BANYAI, M.D., HERMAN HILLEBOE, M.D., J. ARTHUR MYERS, M.D., J. WINTHROP PEABODY, M.D. 480 pp., 78 figs. $9.50. Springfield, Ill.: CHARLES C THOMAS, 1949.

As is true in any volume with chapters written by different authors the standard of excellence of different portions of this book varies considerably. Fortunately the present volume includes among its authors many recognized leaders in their respective fields. Pathologists will find particularly interesting the well-illustrated chapter on The Bacteriology, Infection and Pathology of Tuberculosis, by Sweany and Bogen. The pages on bacteriology are succinctly written and contain a great deal of useful information. Among the other chapters those on Differential Diagnosis, Antibacterial Therapy, and Tuberculosis and the Genitourinary System merit special mention. Also it is of interest to have chapters on the Psychosomatic and on the Public Health Aspects of Tuberculosis included as well as chapters on the relationship between tuberculosis and silicosis, diabetes, and pregnancy respectively. Some of the bibliographies not only furnish a useful index to the recent literature but contain classical references as well.

While recognizing the many excellent parts of the book one may criticize the dictum regarding primary tuberculosis, that "The disease is extremely benign. *Per se* it probably never kills and rarely does it cause significant illness." (Page 42.) Anyone with experience at the postmortem table can give testimony regarding the lethal effects of the tubercle bacillus in cases of progressive primary tuberculosis.

The typography and format of the book are of the high quality expected from the publishers. In general the illustrations are excellent, but the two colored plates leave much to be desired. Only two typographical errors came to the attention of this reviewer, "lumph" on page 83 and "modernately" on page 443. Although written more particularly for physicians interested in the clinical aspects of tuberculosis this book can be recommended for the pathologist as well.

Northville, Michigan C. EUGENE WOODRUFF

Handbook of Diseases of the Skin. By RICHARD L. SUTTON, M.D., Emeritus Professor of Dermatology and Syphilology, University of Kansas Medical School and RICHARD L. L. SUTTON, JR., M.D., Associate Professor of Dermatology and Syphilology, University of Kansas Medical School. 749 pp., 1057 figs. $12.50. St. Louis: The C. V. Mosby Company, 1949.

This newest addition to the series of textbooks by the Suttons is slightly shorter than the fourth edition of their *Introduction to Dermatology* and considerably more extensive than their *Synopsis of Diseases of the Skin.* A comparison of the new book with the last edition of the "Introduction" shows that the text has been compressed, but the number of illustrations has been increased. Comparatively more space has been given to the chapters on anatomy, embryology, physiology, symptomatology and pathology, etiology, diagnosis,

and treatment. The chapters describing specific dermatoses have been rearranged. Dermatoses of known causation including syphilis have been placed first and comprise more than half (403 pages) of the text. Dermatitides of unknown cause follow in a chapter of 65 pages, which seems to indicate considerable progress in our knowledge of the etiology of dermatoses. It must be pointed out, however, that the "unknowns" comprise many of the commonest and most important skin diseases which in practice occupy a much greater share of the dermatologist's time. Chapters on pigmentary disorders, atrophies, malformations and neoplasia, and affections of the cutaneous appendages and the accessible mucous membranes complete the text. A detailed and useful index of 28 pages is added.

Perusal of the text shows that a great amount of information has been condensed in this book. The style is clear and concise, and in most instances the words are so chosen that each one is of importance. The easy readability thus should not deceive the reader who must pay attention to every word and phrase in order to get the full benefit of the information contained. Bibliographic references, also condensed to a small amount of space, are included in the body of the text and invite the interested reader to check up on details. The papers cited are usually chosen for their instructional value and recent date of publication rather than for originality or priority on the subject. Much and well-advised use is made of small print in order to separate basic essential instruction from more detailed information and from the discussion of rarer subjects. The text, more than ever before, reflects the views of the junior author who gives due credit to his father's general influence on his manner of thinking and of treating disease. While the factual information contained in the book is more complete than in some larger texts, the reader is never left in doubt about the personal opinion of the author on controversial subjects. This, as the preface states, "will please some readers and annoy others", and in any case makes for stimulating reading. Considerable space is given to pathology and treatment, and good use is made of tabulation in differential diagnosis. Illustrations are many and usually well chosen photographs, including numerous photomicrographs of skin diseases and particularly of the normal anatomy of the skin. A few exceptions, like the illustration which is mistakenly labelled "Brown-tail moth (Webster)" do not detract from the general excellence of the material.

Altogether, the book confers, in relation to its size, an astonishing amount of up-to-the-minute information on dermatology and syphilology, and does so in well-readable form, enlivened by an abundance of good illustrations and by the personal convictions of the author. It should be valuable not only, or even primarily, to the medical student, but to the dermatologist or other physician seeking concise information on special subjects.

Common Skin Diseases. Ed. 8. By A. C. Roxburgh, M.A., M.D., Emeritus Physician for Diseases of the Skin and Lecturer on Diseases of the Skin, St. Bartholomew's Hospital; Late Editor of the British Journal of Dermatology and Syphilis. 497 pp., 212 illustrations, 8 color plates. $7.00. Philadelphia and Toronto: The Blakiston Company, 1939.

This book is part of a general practice series which does not profess to be a complete text on dermatology and makes only brief mention of some of the less common skin conditions. The early chapters adequately discuss general etiology, signs and symptoms, general diagnosis and general treatment, and conform more or less with similar chapters in most American texts. Under general treatment, the author gives considerable emphasis to the use of Thorium X which has not yet come into general use in this country. His use of solid carbon dioxide is somewhat less conservative than usually recommended, but results seem good.

Discussion of the individual diseases is presented in an orderly fashion. The discussion of pathology of each disease is short, but apparently is in agreement with concepts expressed by American dermatopathologists. The description of signs and symptoms is along conventional lines. Under etiology, many dermatologists would disagree with the concept that seborrhea and seborrheic dermatitis are caused by the pityrosporon of Malassez (*Pityrosporon ovale*), and also, that pityriasis simplex is a form of impetigo which can be "made worse, . . . by east winds". The interesting idea that the itching in scabies is on the basis

of sensitivity to the acarus and may not occur for a month or more after contraction of the infection, is emphasized.

The author uses penicillin extensively in topical form and with less discrimination than its evaluation in this country would indicate. No mention is made of the newer fungicides and fungistatic agents which have come to the fore in recent years, and there is but one scant notation concerning the use of antihistaminics. In general, the book is well written and of interest but it is felt, because of some differences in terminology as well as in the types of medications employed, that most physicians in the United States would be better off to purchase one of the general practice texts on dermatology written in this country.

Detroit JOHN N. GREKIN

Estudos Sôbre a Imunidade Cruzada (Studies on Cross Immunity). By PAULO DE GÓES, Trabalho do Laboratorio de Microbiologia da Faculdade Nacional de Medicina, U. B. 353 pp., 8 tables. Rio de Janeiro: Jornal do Commercio, Rodrigues and Cia, 1947.

The author is in charge of research in the Laboratory of Microbiology of the National Faculty of Medicine of Rio de Janeiro. In this book he reviews at length the evidence from various sources bearing on the antigenic composition of microorganisms and the cross-reactions between various species of bacteria, between bacteria and viruses, molds, protozoa, antigens from the lower and higher animals and from vegetable sources. In the chapter dealing with antigens having similar properties, found in macro- as well as in microorganisms, the author reports his investigations on the Forssmann antigen, Buchbinder's antigen, substance A, and the antiproteus X antibodies of pregnancy and cancer.

The subject matter is carefully arranged and the book should be valuable to workers interested in the field of cross-immunity reactions.

Philadelphia L. M. TOCANTINS

Hindu Medicine. By HENRY R. ZIMMER, Late Visiting Lecturer in Philosophy at Columbia University, Formerly Professor of Sanskrit at the University of Heidelberg. Edited with a Foreword and Preface by LUDWIG EDELSTEIN, PH.D. 203 pp. $4.00. Baltimore: The Johns Hopkins Press, 1948.

This volume represents an incomplete revision of material presented by the late Prof. Henry R. Zimmer in the Hideyo Noguchi Lectureship delivered in 1940 at the Johns Hopkins Institute of the History of Medicine. The work of compiling what is extant of the partially revised lectures presented by this eminent scholar, and from other manuscripts remaining after his death in 1943, was undertaken by Ludwig Edelstein. The Foreword and Preface give a sketch of Zimmer's life and work and his interpretation of Hindu philosophy and Hindu medicine—"the best representative of Hindu earthly life and wisdom".

The material is presented in two parts, extensively annotated by the editor: I. *Medical Tradition and the Hindu Physician* deals with the oldest Vedic traditions, their medical gods, the literary tradition of classic Hindu medicine, the education and career of the physician, and the signs and names of diseases; II. *The Human Body: Its Forces and Resources* discusses the doctrines of life force and breath, the theory of humors and of the body, and Hindu methods for teaching anatomy and physiology.

This book has little bearing on clinical pathology *per se* except for the earnest student of medical history interested in delving into the mystical and allegorical backgrounds of Hindu concepts of health and disease.

Detroit HAROLD C. MACK

Current Therapy 1949, Latest Approved Methods of Treatment for the Practising Physician. By HOWARD F. CONN, M.D., Editor. Consulting Editors: M. EDWARD DAVIS, VINCENT J. DERBES, GARFIELD G. DUNCAN, HUGH J. JEWETT, WILLIAM J. KERR, PERRIN H. LONG, H. HOUSTON MERRITT, PAUL A. O'LEARY, WALTER L. PALMER, HOBERT A. REIMANN, CYRUS C. STURGIS, ROBERT H. WILLIAMS. 672 pp. $10.00. Philadelphia and London: W. B. Saunders Company, 1949.

This compendium of treatment is an outstanding contribution to the field of general therapeutics. Dr. Conn and his twelve consulting editors have contrived to make this text of inestimable value as a treatment reference. Over 250 clinical entities and their specific treatment are discussed in fourteen well-organized sections. Clarity and brevity are keynotes of the book. Practically every important clinical entity in each specialty field is discussed from the standpoint of treatment.

Although there is little of interest to the clinical pathologist, *Current Therapy* should hold a formidable place in the therapeutic armamentarium of the practicing physician.

Detroit MARK DALE

Practical Aspects of Thyroid Disease. By GEORGE CRILE, JR., M.D., F.A.C.S., Department of Surgery, Cleveland Clinic, Cleveland, Ohio. 355 pp., 101 figs. $6.00. Philadelphia, Pennsylvania: W. B. Saunders Company, 1949.

Dr. Crile states in his preface to this book that the treatment of diseases of the thyroid gland had become well accepted as a surgical problem, until the past few years, when with the discovery of effective antithyroid drugs, it again became controversial whether or not hyperthyroidism is better treated by conservative (medical) management. The possibility is also considered that in the future the radiologist may also play an ever-increasing part in the treatment of diseases of the thyroid gland with radio-active iodine. With these considerations as the background, the author has written the book, based upon his personal experiences in the surgical treatment of 1000 patients with diseases of the thyroid gland and from observations made on several hundred patients treated with antithyroid drugs in the section on endocrinology of the Cleveland Clinic. The book is intended for both medically and surgically trained readers, so that each may understand better what may be accomplished by the internist, the radiologist, or the surgeon.

The contents are divided into twenty-seven chapters and a conclusion. It considers first the physiology of the thyroid gland incorporating the newer knowledge of the role of the anterior pituitary thyroid-stimulating hormone and the action of the thyroid hormone on the pituitary. The action of thiouracil is briefly discussed. The following chapters discuss the pathology, prevention and treatment of endemic goiter. Chapter IV outlines the pertinent facts concerning intrathoracic goiter. This is followed by a discussion of hyperthyroidism, use of antithyroid drugs, roentgen radiation and the radioactive iodine in the treatment of hyperthyroidism. Discussions of preoperative management, anesthesia for thyroidectomy, and the technic of thyroidectomy follow. Next he considers postoperative care, complications and recurrent hyperthyroidism. The discussions of the nodular types of goiter are interjected in the chapters on hyperthyroidism. After discussing congenital anomalies the book concludes with a well rounded review of carcinoma of the thyroid and thyroiditis. The discussion of papillary carcinoma of the thyroid is most complete. The author now concludes that most of the papillary carcinomas originate in the thyroid and appear in the cervical region as metastasis to the cervical lymph nodes. Some of these present metastatic involvement before a neoplasm is noted in the thyroid gland; although probably all have their origin there. The peculiarities of these growths are noted, especially their tendency to remain localized to the cervical nodes and the usual successful surgical treatment even after recurrent metastasis. Dr. Crile divides thyroiditis into: (1) subacute (pseudotuberculous giant cell) thyroiditis, (2) struma lymphomatosa (Hashimoto's thyroiditis), and (3) Riedel's struma (woody or ligneous thyroiditis). Each type is a distinct entity and, in his opinion, one does not lead to the other. There are also types of thyroiditis which fail of classification.

The book constitutes a concise authoritative review of thyroid diseases in the light of modern knowledge.

Detroit D. C. BEAVER

SELECTED ABSTRACTS

Primary Plasma Cell Tumors of the Upper Air Passages and Oral Cavity. A. P. STOUT, AND F. R. KENWAY. Cancer, **2:** 261–278, 1949.

The authors divide plasma cell tumors into six categories: (1) plasma cell granuloma, (2) benign localized plasmacytoma, (3) malignant solitary tumor, (4) malignant tumor with generalization of metastases, (5) plasma cell myeloma, (6) plasma cell leukemia. Detailed histories of 8 patients are presented, and an analysis of the literature on primary plasma cell tumors of the nasopharynx is reported. These data indicate that the condition is four times as frequent in men as in women and that three-fourths of the cases develop in the fifth, sixth and seventh decades of life. Tables are included showing principal sites of involvement and location of metastases. In a final table, all cases (104) in the literature are divided into 15 groups with an analysis of treatment, complications and survival periods. A complete bibliography is appended.

Brooklyn LEO M. MEYER

The Occurrence of False Positive Trichina Precipitin Tests in Infectious Mononucleosis. F. A. BASSEN, A. E. THOMSEN, AND A. SILVER. J. Lab. and Clin. Med., **34:** 543–548, 1949.

Precipitin tests for trichinosis were performed on 5 patients admitted to the hospital with signs and symptoms suggestive of the disease. Elevated titers were found in all instances. Coincidental or subsequent heterophile agglutination tests and repeated blood counts indicated that all of these patients were suffering from infectious mononucleosis.

The precipitin test for trichinosis has long been the subject of much controversy since it may be positive in other parasitic infections and in periarteritis nodosa. Confirmatory tests, such as muscle biopsy and complement-fixation tests should be performed in order to prove the diagnosis in all suspected cases.

LEO M. MEYER

Hematologic Observations in Patients with Chronic Hepatic Insufficiency. T. JARROLD, AND R. VILTER. J. Clin. Investigation, **28:** 286–292, 1949.

Thirty patients with portal cirrhosis uncomplicated by recent hemorrhage were studied for changes in the morphology of the peripheral blood and bone marrow. Twenty-four of the patients were anemic and of this number 20 had a macrocytosis and the remaining four had a normocytic anemia. The leukocytes showed none of the changes associated with typical Addisonian anemia. The bone marrow was hypocellular in 9 patients and normally cellular in the remaining 21. Only 3 patients had evidence of a megaloblastic arrest but a history of dietary insufficiency (extrinsic factor) could be elicited. Normoblasts were present in increased numbers in most of the patients. Of greater significance was the plasmacytosis of the bone marrow which reached 23 per cent and seemed to bear a relationship to the serum hyperglobulinemia. Seven patients with biliary cirrhosis and 7 with acute hepatitis failed to show any correlation of plasma cells in the bone marrow and the amount of serum globulin.

These data are of particular interest as this reviewer was stimulated by the authors of this paper to study the bone marrow of patients with chronic infectious disease associated with hyperglobulinemia. The incidence of plasmacytosis was extremely low in contradistinction to the results reported above in chronic hepatic insufficiency.

LEO M. MEYER

A Simple Quantitative Calcium-Formol Gel Reaction. B. A. VERHAGEN. Acta med. Scandinav., **132:** 265–282, 1948.

A simple quantitative method is described for determination of the gamma globulin and euglobulin of the serum using various dilutions of serum, saline, formalin and calcium chloride. The procedure resembles the simple formol gel technic in use in this country. The

modifications presented by this author make this an easy procedure which can be performed in any hospital laboratory. No special equipment is necessary. The results obtained have been confirmed by electrophoresis and chemical analysis.

LEO M. MEYER

Action of Furacin in Delaying Growth of a Transplanted Fibrosarcoma in Mice. M. N. GREEN AND C. F. FRIEDGOOD. Proc. Soc. Exper. Biol. and Med., **69**: 603–604, 1948.

The antibacterial compound furacin (5-nitro-2-furaldehyde semicarbazone) was found to inhibit enzymes participating in glucose and pyruvate metabolism. The effect of this antimetabolite on tumor growth was studied in C_3H mice with sarcoma 13 transplants. Ten of the 40 mice used served as controls; the remaining animals were divided into 3 groups of 10 each, and received different doses of furacin before and after inoculation of tumor. All animals eventually developed tumors and died from them. However, definite inhibition of tumor-growth was observed in the furacin-treated animals. In one group this was expressed in prolongation of the latent period of the transplants, and in all groups the life span of mice injected with furacin significantly exceeded the life span of the controls. The tumors of the treated animals were reported to have shown evidence of cellular degeneration that was absent in control tumors. The adrenals of the furacin animals presented hypertrophy of the fascicular zone.

Chicago KURT STERN

Aldolase in the Serum and Tissues of Tumor-Bearing Animals. J. A. SIBLEY AND A. L. LEHNINGER. J. Nat. Cancer Inst., **9**: 303–309, 1949.

Warburg and Christian (Biochem. Ztschr. **314**: 399, 1943) found a considerable increase of aldolase (zymohexase) in the serum of rats with Jensen sarcoma. Aldolase is a glycolytic enzyme, catalyzing the cleavage of hexose diphosphate into 2 moles of 3-carbon compounds (glyceraldehyde phosphate and dihydroxyacetone phosphate). It is present in muscle, many other organs, and serum.

The authors of this study devised a new accurate method of assay of aldolase, and determined the enzyme activity in serum of normal rats and of animals with transplanted tumors (sarcoma 39, Walker carcinosarcoma). They corroborated Warburg's findings of a marked rise of the aldolase level in the serum of tumor-bearing rats; the enzyme activity approximately paralleled the size of the tumor. Complete removal, or spontaneous regression, of tumors was followed by a prompt return of the serum aldolase to normal levels. On the other hand, the enzyme activity in the serum of tumor-free animals was not changed by starvation, anemia, pregnancy, liver damage, or liver regeneration. Livers of tumor-bearing animals contained significantly higher enzyme concentrations than livers of normal rats; no appreciable difference in the enzyme level was found in other organs of tumor-free and tumor-bearing rats. The tumors contained enzyme concentrations of a similar range as brain and heart, organs which were surpassed in aldolase activity only by skeletal muscle.

The aldolase level in normal human blood serum was found to be somewhat lower than in rat serum. Twenty-five out of 104 serums of cancer patients exhibited a definite increase of the enzyme level. The low incidence of the enzymatic change and the absence of any correlation between enzyme level and clinicopathologic findings preclude practical application of aldolase determinations to the management of human cancer. The significance of the findings lies rather in the demonstration of a quite specific enzymatic deviation, involved in carbohydrate metabolism and apparent in serum and liver of tumor-bearing animals.

KURT STERN

Action of a Fungus (Streptothrix felis D.A.) on Mouse Cancer. G. DE ANGELIS. Oncologia, **2**: 43–62, 1949.

In earlier experiments (1936) the author had observed regression of mouse tumors following infection of animals with a streptothrix strain (originally obtained from a granuloma of a domestic cat). The present report deals with the effect of a partially purified filtrate of

streptothrix cultures on the Ehrlich mouse adenocarcinoma. Intraperitoneal injections of the filtrate brought about regression of the transplanted tumors in over 90 per cent of 330 treated animals. The animals remained free of recurrence and were resistant to a subsequent second inoculation of the same tumor. On the other hand, all 286 control animals succumbed to progressive tumor growth. The filtrate was found effective in small as well as in far-advanced tumors; within the latter group, controls were said to "survive only for a few days" while 87 per cent of the injected animals were cured. Histologic study of tumors at various intervals following injection, revealed progressive cellular degeneration with karyorrhexis and karyolysis, infrequent petechial hemorrhages, and peripheral lymphocytic infiltration. Eventually, the entire tumor was transformed into amorphous debris. In advanced tumors liquefaction necrosis set in rapidly, and large amounts of sudanophilic granules were demonstrable in the fluid. Resorption of the necrotic material then ensued, and was stated not to have impaired the general well-being of the animals. On the basis of these results, the author felt justified in classifying the streptothrix filtrate as an "anticarcinomatous antibiotic."

A few critical remarks appear in order: (1) The statement that the white mice used by the author are to be considered a "pure strain" because they were all derived from a single pair of stock albino mice, is untenable on genetic grounds; hence this work was subject to the vagaries of tumor growth often apparent in heterozygous animals. (2) No data as to weight curves of treated animals were presented; if the admittedly toxic filtrate interfered with food intake and assimilation, this secondary effect would have to be taken into consideration. (3) Finally, it is regrettable that no clear-cut technical data on the mode of culture of the fungus and on the "purification" of the filtrates were presented. Such data would be essential in order to facilitate re-examination of the results of the Italian author.

KURT STERN

Purine Metabolism In Tetrahymena and Its Relation to Malignant Cells in Mice. G. W. KIDDER, VIRGINIA C. DEWEY, and R. E. PARKS, JR. Science, **109:** 511–514, 1949.

Extensive studies had been carried out previously on the purine and pyrimidine metabolism in a protozoon (*Tetrahymena gelei*) as compared with mammalian cells. In contrast to the latter, the protozoan cells are unable to synthesize guanine and, therefore, require this purine in their nutriment. A number of substituted purines and pyrimidines, prepared by Hitchings and co-workers, were tested for interference with nucleic acid synthesis. In accord with the inability of tetrahymena to synthesize guanine, the guanine analogue, guanazolo (5-amino-7-hydroxy-1 H-v-triazolo(d)pyrimidine), proved to be a highly effective antimetabolite for this microorganism.

Normal mammalian cells apparently are not harmed by the compound, since its administration to mice proved nontoxic. This is again in harmony with the earlier findings that mammalian cells are able to synthesize guanine, and hence are not affected by the competitive action of the guanine analogue. On the other hand, the cells of a transplanted mouse adenocarcinoma proved to be highly susceptible to the administration of guanazolo, complete cessation of tumor growth resulting from doses of 0.5 mg. of the purine antagonist injected twice daily. The authors assumed that, in contrast to normal mammalian cells, the mouse tumor cells had lost the ability of synthesizing guanine, thus resembling the protozoon tetrahymena in their metabolism. When the injections of guanazolo were discontinued, tumor growth was resumed shortly afterwards, and histopathologic study also showed that the treatment had not resulted in death of the cancer cells. The growth-checking effect of guanazolo was found to extend equally to spontaneous mammary carcinomas of C_3H mice, and to a lymphoid leukemia of strain A animals.

Chicago KURT STERN

NEWS AND NOTICES

Alabama Association of Pathologists

The Alabama Association of Pathologists recently elected the following officers for the year 1949-1950: President, Dr. Joseph A. Cunningham, Birmingham; Vice President, Dr. James S. P. Beck, Tuscaloosa; Secretary-Treasurer, Dr. Albert E. Casey, Birmingham; and Member of Executive Committee, Dr. Roger D. Baker, Birmingham.

The annual meeting of the association was held April 21, 1949, in Montgomery, Alabama, at the Veterans Hospital. Dr. Emmerich von Hamm conducted the annual slide seminar at this meeting, and Dr. G. W. Millett and Dr. C. R. Lafferty of Veterans Hospital were hosts to the association.

Thick Film Diagnosis of Malaria

The following notice has been submitted by R. D. Lillie, M.D., Chief, Laboratory of Pathology and Pharmacology, U. S. Public Health Service, Bethesda, Md.

"At the April 16 meeting of the Biological Stain Commission, the Board of Trustees voted that hereafter Giemsa stain will be certified in two varieties, namely, 'Giemsa stain, Azure B Type, for malaria and blood work' and 'Giemsa stain, Azure A Type, for hematology and bacteriology'.

"The Azure B type closely resembles tinctorially and spectroscopically the Grübler and Hellborn Giemsa stains of the 1930's and is the variety especially recommended to give the faintly greenish blue tint to parasite cytoplasm which contrasts well with the grayish or greenish blue background of the thick film stained at pH 7.0. This is recommended by many malariologists for thick film work.

"The Azure A type gives darker red chromatin stains, grayer or more violet blue lymphocyte cytoplasm, and perhaps somewhat heavier staining of micro-organisms. Its useful life is probably shorter under average tropical storage conditions. It is preferred by many American hematologists."

American Association of Blood Banks

The second annual meeting of the American Association of Blood Banks will convene in Seattle, Washington, at the Olympic Hotel, November 3 to 5, 1949.

The program committee consists of Dr. Julius W. Davenport, Jr., Southern Baptist Hospital, New Orleans, Louisiana, Chairman; Dr. Paul I. Hoxworth, Cincinnati General Hospital, Cincinnati, Ohio; Mr. Charles G. Ransom, 1911 Broadway, Nashville, Tennessee; Dr. Joseph Porter, Maine General Hospital, Portland, Maine; and Dr. William Levin, John Sealy Hospital Blood Bank, Galveston, Texas.

Dr. Ralph G. Stillman, 315 East 68th Street, New York, New York, is President of the Association, and Marjorie Saunders, 3301 Junius Street, Dallas 1, Texas, is Secretary.

Personal

Dr. F. William Sunderman, previously of the University of Pennsylvania, resigned from his position as Head of the Department of Clinical Pathology at the Cleveland Clinic on July 1st to accept the professorship of Clinical Pathology and Experimental Medicine at the University of Texas and the directorship of Clinical Research and of the Department of Clinical Pathology at the M. D. Anderson Hospital in Houston, Texas. Dr. Bradley E. Copeland and Dr. John B. Fuller also resigned from the Cleveland Clinic and will be associated with Dr. Sunderman as assistants in his department.

Pennsylvania Association of Clinical Pathologists

A joint meeting of the Pennsylvania Association of Clinical Pathologists, The College of American Pathologists, Middle Eastern District, the Clinical Pathological Society of

Pittsburgh, and the Allegheny County Division of American Cancer Society, Inc., will be held in Pittsburgh on Friday, September 23, 1949, at the Roosevelt Hotel. On the following day, September 24, the regular semi-annual scientific meeting of the Pennsylvania Association of Clinical Pathologists will take place. A feature of the joint meeting will be a Slide Seminar on "Cytologic Diagnosis of Cancer of the Lung" with Dr. Peter A. Herbut of Philadelphia acting as referee.

The Secretary of the Association is Dr. Henry F. Hunt, Red Lane, Danville, Pa.

Minnesota Society of Clinical Pathologists

The Annual Meeting of the Minnesota Society of Clinical Pathologists was held at the Minnesota Club in St. Paul on Wednesday, May 11, 1949. The following officers were nominated and elected: President, A. H. Wells; Vice President, John F. Noble; and Councilor, John McDonald. The meeting was adjourned to hear the A. H. Sanford Lecture by Dr. Leonard W. Larson of Bismarck, N. D., on "The Relation of the Pathologist to the General Practitioner" before the General Session of the Minnesota State Medical Association.

The annual dinner honored Dr. E. T. Bell at the Minnesota Club. Forty members and junior members, and four guests and honorary members were present.

Western Missouri Society of Pathologists

The Western Missouri Society of Pathologists was formed February 28, 1949 in Kansas City, Missouri under the leadership of Dr. Victor Buhler, Counselor for the Western Missouri District. The following officers were elected for the year 1949: President, Russel W. Kerr and Secretary-Treasurer, Jack H. Hill.

Refresher Courses in Laboratory Diagnosis

The Communicable Disease Center of the Public Health Service is offering the following refresher courses:

Laboratory Diagnosis of Parasitic Diseases, 6 weeks, from September 12 to October 21, 1949;

Laboratory Diagnosis of Bacterial Diseases, Part 2, General Bacteriology, 5 weeks, from October 31 to December 2, 1949;

Laboratory Diagnosis of Rabies, 1 week, from November 14 to November 18, 1949;

Advanced Enteric Bacteriology, 2 weeks, from December 5 to December 16, 1949.

Information and applications should be requested from the Chief, Laboratory Division, Communicable Disease Center, 291 Peachtree Street, N.E., Atlanta, Georgia.

TECHNICAL SECTION

CO-THROMBOPLASTIN ASSAY

A Means of Study of Abnormalities of Blood Coagulation*

FRANK D. MANN, M.D.

From the Division of Clinical Laboratories, Mayo Clinic, Rochester, Minnesota

The Quick prothrombin time depends upon several different factors in the plasma.[3–5] Seegers and Ware[6] have brought forward impressive evidence that only one of these, prothrombin, is converted into thrombin. The others, which may be termed prothrombin conversion factors, affect the rate of this conversion. While for general clinical purposes it is perhaps desirable to test for these factors collectively, it would also be desirable to assay each one individually. Ware and Seegers[7] have described a determination of "ac-globulin" based on the rate of conversion of purified prothrombin to thrombin. The two-stage method[8] appears to be a good measure of prothrombin itself, provided conditions are so arranged that conversion of thrombin is complete. With the two-stage system employed in this laboratory[2] a striking initial lag in thrombin formation has been consistently observed. Thus only traces of thrombin are formed in the first minute of incubation† with thromboplastin; during the second minute more than ten times as much thrombin is formed as in the first minute (Table 1). This would seem to indicate a preliminary reaction, which might afford a useful assay procedure.

The procedure finally adopted was as follows: All reagents were prepared as for the two-stage procedure previously described.[2] One milliliter of thromboplastin (which includes calcium) was *pre-incubated at room temperature with* 0.5 ml. of plasma or serum made up to an appropriate dilution (at least 1:100 for normal plasma) with imidazole buffer of pH 7.2. At exact intervals, usually multiples of three minutes, 0.15 ml. of this mixture was removed and blown into 0.05 ml. of a 1:25 dilution of defibrinated normal plasma. After exactly one minute 0.2 ml. of fibrinogen-acacia solution was blown into this second mixture and the clotting time was determined just as in the two-stage method. In this way the composition of both incubation and clotting mixtures was kept the same as in the two-stage determination. The observed clotting times were measures of the amounts of thrombin formed in one minute from the aliquot of normal plasma 1:25 under the influence of thromboplastin which had been subjected to the specified pre-incubation treatment. These amounts were large compared to the one-minute yield with the same thromboplastin without pre-incubation and also large compared to the yield obtained from the small amount of plasma used for pre-incubation without added plasma 1:25. (It should be noted

* Received for publication, May 13, 1949.

† The term "incubation" has been widely used in literature on the two-stage method to denote the treatment of prothrombin with thromboplastin.

that the one-minute thrombin yield was usually less than that shown in Table 1, often quite unassayable, and that conversion of the small amount of prothrombin in the very dilute plasma used for pre-incubation was incomplete in three minutes.) Hence the "one-minute thrombin yield" under the foregoing conditions was principally a measure of a reaction between the thromboplastin and the material used for pre-incubation. For the present, this effect will be referred to as the "co-thromboplastin activity" of the material tested and the test procedure as the "co-thromboplastin assay".

Table 2 shows an example of results obtained; the plasma and serum for pre-incubation as well as the plasma 1:25 were all from the same normal person. Within rather narrow limits, the one-minute thrombin yield is approximately a linear function of the amount of plasma used for pre-incubation and of the time

TABLE 1

THROMBIN FORMATION IN THE TWO-STAGE SYSTEM

TIME OF INCUBATION, MINUTES	CLOTTING TIME, SECONDS	THROMBIN UNITS PER ML. INCUBATION MIXTURE
1	67	0.14
1½	26	0.9
2	17	2.0
2½	16	2.25
3	15	2.5

TABLE 2

ONE-MINUTE THROMBIN YIELDS, IN THROMBIN UNITS, AFTER PRE-INCUBATION FOR THE SPECIFIED TIMES WITH THE SPECIFIED MATERIALS. PLASMA PROTHROMBIN 340 UNITS/ML. SERUM PROTHROMBIN <1 UNIT/ML.

TIME OF PRE-INCUBATION, MINUTES	PLASMA 1:200	PLASMA 1:100	SERUM 1:200	SERUM 1:100	HEATED SERUM 1:100
3	0.6	1.35	1.35	2.25	0.9
6	1.2	2.5	2.25	2.5	1.6
9	2.0	2.5	2.5		

of pre-incubation. When both an unknown and a control normal plasma are pre-incubated for three minutes with the same thromboplastin, which is then added to the control plasma 1:25, the co-thromboplastin activity of the unknown can be calculated as follows:

$$\frac{\text{One-minute thrombin yield of unknown}}{\text{One-minute thrombin yield of control}} \times \frac{\text{Dilution of unknown}}{\text{Dilution of control}} \times 100 = \% \text{ co-thromboplastin activity}$$

Serum has distinctly more co-thromboplastin activity than the plasma from which it is derived, although prothrombin is virtually absent. Considerable co-thromboplastin activity remained in serum heated for thirty minutes at 56 C. in

1:100 dilution in imidazole buffer of pH 7.2; such heat treatment of undiluted serum almost completely inactivated it. Treatment with zymin and with ammonia, which had been previously[5] found to destroy prothrombin conversion activity, did not appreciably affect co-thromboplastin activity. No significant association of co-thromboplastin with platelets was observed.

A few examples of results on abnormal plasma are given in Table 3. Aging of plasma (two months at 5 C., then two years at −20 C.) did not seem to affect co-thromboplastin activity. In a patient with very severe hepatic damage co-thromboplastin and prothrombin were decreased to about the same extent.

In patients on routine Dicumarol therapy, especially those in whom a marked effect was rapidly developing, co-thromboplastin was often decreased very much more than prothrombin. Hurn, Barker and Mann[1] observed in 1947 that early in the course of Dicumarol therapy the Quick prothrombin time was affected much more than the two-stage prothrombin level. This difference becomes much more striking when 0.1 ml. of human serum, virtually free of prothrombin,

TABLE 3

CO-THROMBOPLASTIN ASSAYS ON ABNORMAL PLASMA

TYPE OF PLASMA	QUICK PROTHROMBIN TIME		TWO-STAGE PROTHROMBIN		CO-THROMBOPLASTIN, % OF CONTROL
	Seconds	% normal*	Units/ml.	% average normal	
Aged (*in vitro*)	162	<5	300	91	132
Severe hepatitis, coma	62	10	29	9.1	7.5
Dicumarol	86	8	110	34	3.9

* From dilution curve.

is added to the two-stage system, which greatly increases the yield of thrombin from Dicumarol plasma, although not from normal plasma. It seems quite possible that the principal effect of Dicumarol may be on co-thromboplastin rather than on prothrombin; in any event the Dicumarol effect is complex and will be thoroughly restudied with newer technics.

Co-thromboplastin assay is not recommended for routine clinical use at present. It should be feasible for any laboratory which can perform the two-stage procedure, but it cannot be predicted that the co-thromboplastin test could necessarily be done with reagents other than those indicated. It is hoped that the technic may prove useful in the investigation of abnormalities of blood coagulation.

SUMMARY

Pre-incubation of thromboplastin with very dilute serum or plasma greatly increases the initial rate of thrombin formation when plasma is added. An assay of this co-thromboplastin activity is described; it often shows far greater decreases after administration of Dicumarol than does the two-stage prothrombin determination.

REFERENCES

1. HURN, M., BARKER, N. W., AND MANN, F. D.: Variations in prothrombin and antithrombin following the administration of Dicumarol. Am. J. Clin. Path., **17**: 712–718, 1947.
2. HURN, M., AND MANN, F. D.: The determination of prothrombin (two-stage method) and antithrombin using commercially available reagents. Am. J. Clin. Path., **17**: 741–746, 1947.
3. MANN, F. D., AND HURN, M.: Relation of complement to blood coagulation. Proc. Soc. Exper. Biol. and Med., **67**: 83–85, 1948.
4. MANN, F. D., HURN, M., AND MAGATH, T. B.: Observations on the conversion of prothrombin to thrombin. Proc. Soc. Exper. Biol. and Med., **66**: 33–35, 1947.
5. QUICK, A. J.: Discussion. In: Transactions of the First Conference on Blood Clotting and Allied Problems. New York: Josiah Macy, Jr. Foundation, pp. 116–125, 1948.
6. SEEGERS, W. H., AND WARE, A. G.: Activation of purified prothrombin with sodium citrate. Federation Proc., **8**: 249, 1949.
7. WARE, A. G., AND SEEGERS, W. H.: Plasma accelerator globulin; partial purification, quantitative determination and properties. J. Biol. Chem., **172**: 699–705, 1948.
8. WARNER, E. D., BRINKHOUS, K. M., AND SMITH, H. P.: A quantitative study on blood clotting; prothrombin fluctuations under experimental conditions. Am. J. Physiol., **114**: 667–675, 1936.

RECENT ADVANCES IN THE CULTURE DIAGNOSIS OF FUNGUS DISEASE*

M. L. LITTMAN, PH.D., ALBERT L. McQUOWN, M.D., AND JOHN D. SCHNEIDAU, M.S.

From the Department of Tropical Medicine and Public Health, Tulane University School of Medicine, the Department of Pathology, Charity Hospital and the Department of Biology, Loyola University, New Orleans, Louisiana

INTRODUCTION

During the past several years a number of new culture mediums for the isolation of pathogenic fungi have appeared. Bernhardt[1] in 1944 reported a rice-and-water medium containing crystal violet (in a dilution of 1:385,000) for the cultivation of dermatophytes. The following year Leise and James[5] reported an alkaline agar which inhibited saprophytic fungi but not dermatophytes. Shortly afterwards, Thompson[11] described a "hormone" blood agar containing penicillin and streptomycin which inhibited bacteria but permitted fungi to develop. Recently Boeing and Laffer[2] reported a similar medium containing a higher concentration of penicillin and streptomycin.

In 1947 a departure from the usual type of culture medium for fungi was made by Littman[6] who described an oxgall (bile) agar medium which permitted saprophytic and pathogenic fungi plated thereon to grow as small, well-separated, *non-spreading* colonies. Crystal violet was incorporated in a final concentration of 1:100,000 to restrict the growth of staphylococci and other gram-positive bacteria. Streptomycin was present in the amount of 30 micrograms (units) per ml. of agar medium to restrict the growth of gram-negative bacteria. It was possible to inoculate the new agar heavily with all types of clinical specimens, such as sputum, feces, exudates, hair, skin and nail scrapings. The growth of both gram-positive and gram-negative bacteria was restricted while all varieties of fungi grew promptly as small, discrete, *non-spreading* colonies easy to isolate in pure culture. Species of nocardia, however, failed to grow because of their sensitivity to streptomycin.

Littman[7] demonstrated that pure cultures of fungi began to proliferate in approximately the same time on his medium as on Sabouraud's dextrose agar. He also noted that highly diluted, pure culture suspensions of fungi grew with equal speed and with equal sensitivity on his medium as on Sabouraud's agar. When tested with 47 clinical specimens, however, the new medium detected 3 times as many species of fungi and 4 times as many pathogenic fungi as did Sabouraud's medium. The promising features of the medium for diagnosis and research were reported.[7]

The present report concerns experiences with Littman's oxgall agar as used experimentally and routinely for 12 months in the Diagnostic Laboratory and Dermatology Clinic at Charity Hospital, New Orleans, Louisiana, for the diagnosis of cutaneous and systemic mycoses.

* Received for publication, March 12, 1949.

CUTANEOUS MYCOSES

Method

The subjects were patients reporting to the Dermatology Clinic who were thought to have cutaneous mycoses. The affected area of the skin was exposed, cleansed with acetone and then scraped lightly with a Bard-Parker knife until the edge of the blade had collected a fine powdery deposit of epidermal tissue. Approximately equal quantities of scrapings were than streaked over Littman's oxgall agar and Sabouraud's dextrose agar with the flat side of the knife blade. Potassium hydroxide mounts were not routinely made. Plates were incubated at room temperature (25 C.) in a metal cabinet fitted with an open pan of water which provided a favorable humidity. Plates were examined in five days, then held for seven more days before they were discarded. Colonies of fungi thought to be dermatophytes were counted and transplanted to stock culture agar slants (nutrient agar enriched with 0.2 per cent tryptone and 1 per cent dextrose). Slide cultures were made for verification of species with potato dextrose agar[4, 8] in order to force sporulation.

Results

Of the total of 222 patients examined, there were 11 white males, 22 white females, 71 Negro males and 118 Negro females. Fifty-seven patients or 25 per cent of the total number, carried pathogenic fungi, as outlined in Table 1. While 57 cases of dermatomycoses were detected with Littman's oxgall agar, only 10 were detectable with Sabouraud's agar probably due to overgrowth of saprophytic fungi and bacteria. Of the 21 Microsporum infections discovered, all were in children and all involved the scalp. The predominant organism was *Microsporum audouini*, the epidemic species of human ringworm. Of 20 children infected with this organism, all were of the Negro race, the average age was 5.3 years and the ages varied from one month to 14 years. The infection was found 9 times as frequently in boys as in girls. This ratio is the same as that observed in the Minneapolis and St. Paul epidemic of ringworm in 1945 and 1946, reported by Steves and Lynch.[10]

Of the 35 Trichophyton infections discovered, the majority occurred in adults, 14 in females and 21 in males. The average age was 30.3 years and the ages ranged from 8 to 60 years. The predominant organism was *Trichophyton rubrum* and the majority of infections was limited to the inguinal region, abdomen, buttocks or back. Other regions of the body less frequently involved were the hands, axillae, legs and feet. One *Epidermophyton floccosum* infection of the inguinal region and 2 *Trichophyton sulfureum* infections, one of the scalp and one of the foot, were observed.

Negro males comprised the group most susceptible to fungus infection of the body and scalp (see Table 2).

At this point in the study of cutaneous mycoses, the use of Sabouraud's dextrose agar for primary isolation of fungi was discontinued. Henceforth, Littman's oxgall agar was used alone for examination of 325 additional patients. The com-

TABLE 1

RELATIVE EFFECTIVENESS OF CULTURE MEDIUMS IN DETECTING CASES OF CUTANEOUS MYCOSES IN 222 PATIENTS

CAUSATIVE ORGANISMS	SABOURAUD'S DEXTROSE AGAR	LITTMAN'S OXGALL AGAR	NEGATIVE ON LITTMAN'S AND POSITIVE ON SABOURAUD'S MEDIUM	POSITIVE ON LITTMAN'S AND NEGATIVE ON SABOURAUD'S MEDIUM
M. audouini (human)	7	20	0	13
M. gypseum (fulvum)	0	1	0	1
T. rubrum	3	33	1	29
T. sulfureum (crateriform)	0	2	0	2
E. floccosum	0	1	0	1
Total cases detected	10	57	1	46

TABLE 2

DERMATOMYCOSES BY SEX AND RACE

RACE AND SEX OF PATIENTS	NUMBER EXAMINED	SCALP INFECTION		BODY INFECTION	
		Number of Cases	Per cent	Number of Cases	Per cent
White males	11	0	0	2	18
White females	22	0	0	4	17
Negro males	71	19	26	19	26
Negro females	118	2	1	11	9
Totals	222	21	9	36	16

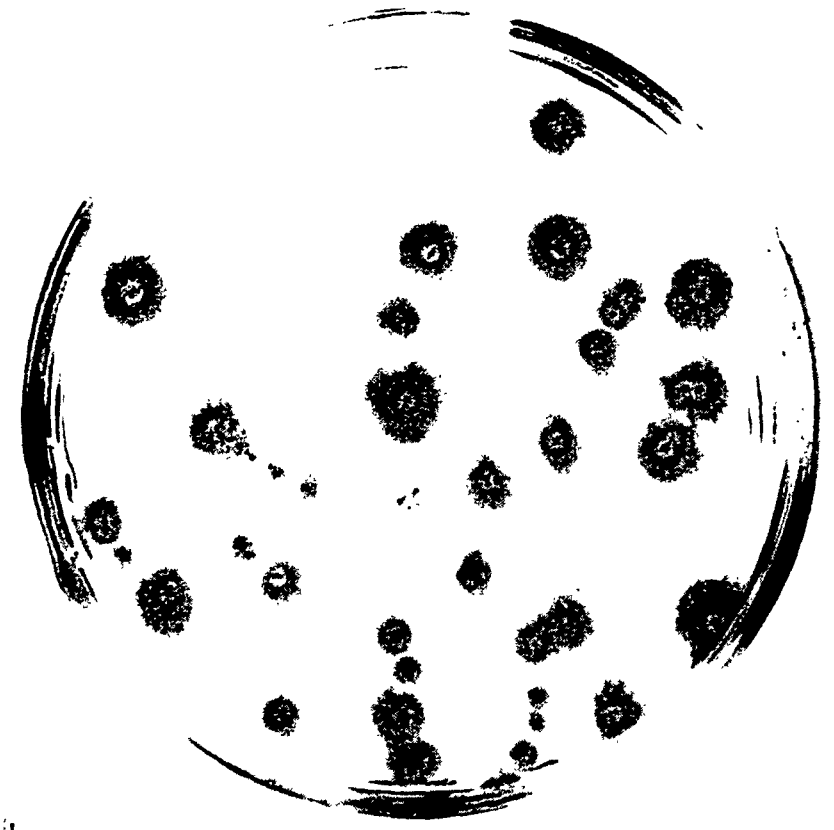
TABLE 3

CASES OF DERMATOMYCOSES DISCOVERED IN 547 PATIENTS EXAMINED JULY, 1947 TO JULY, 1948, USING LITTMAN'S OXGALL AGAR

CAUSATIVE ORGANISMS	NUMBER	PER CENT
M. audouini (human)	84	52.5
M. canis (lanosum, animal)	3	1.8
M. gypseum (fulvum)	1	0.6
T. rubrum	58	36.3
T. sulfureum (crateriform)	3	1.9
T. mentagrophytes (gypseum)	10	6.3
E. floccosum	1	0.6
Total cases detected	160	100

bined results are presented in Table 3. Of 547 patients with demonstrable skin lesions which were scraped, 160 were found to carry pathogenic skin fungi. Approximately half of the cases of dermatomycoses were due to *M. audouini*.

After the new culture medium had been in use in the laboratory for only a



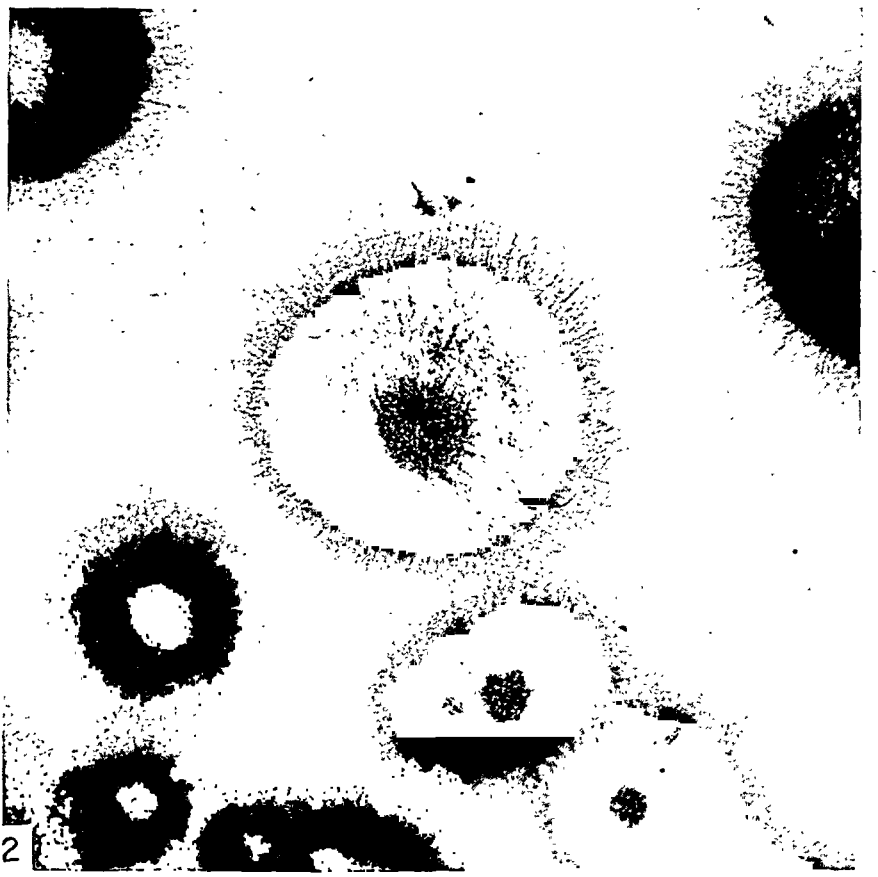
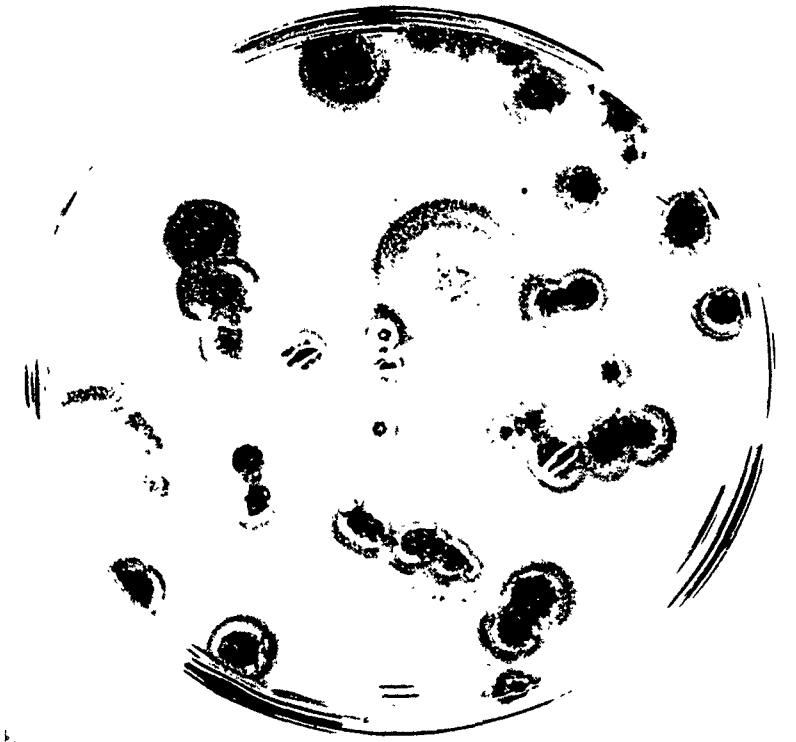


FIG. 1. Case 2. Culture of scalp scrapings streaked on Littman's oxgall agar. The skin was first cleansed with acetone. A pure culture of *Microsporum audouini* was obtained.

FIG. 2. Colonies of *Microsporum audouini* on Littman's oxgall agar showing discrete growth. × 6. Army Institute of Pathology, Acc. No. 218120-S10



3



4

FIG. 3. Numerous colonies of *Trichophyton rubrum* isolated from the inguinal region, previously cleansed with acetone. Note the pigmented margins of the colonies. Littman's oxgall agar.

FIG. 4. Pure culture of *Trichophyton mentagrophytes* (gypseum) isolated from the feet, after cleansing the skin with acetone. Littman's oxgall agar.

short period of time, it became a relatively simple task to detect plates carrying dermatophytes. Almost without exception the colonies of pathogenic fungi were very numerous, often covering the surface of the agar and frequently appearing in pure culture (see Figs. 1–4). On Sabouraud's agar, colonies of dermatophytes were few in number and were frequently closely enmeshed with luxuriant growth of saprophytic fungi.

SYSTEMIC MYCOSES

Over a 12 month period exudates were examined from 162 cases in which a differential clinical diagnosis was required. The specimens were streaked on Sabouraud's dextrose agar and Littman's oxgall agar. Nine cases of systemic mycoses were found as follows: Two cases each of sporotrichosis and North American blastomycosis; one case each of cryptococcosis and coccidioidomycosis, and 3 cases of moniliasis. The relative effectiveness of the 2 culture mediums is shown in Table 4. Three cases of actinomycosis were found using veal infusion agar. Of

TABLE 4

RELATIVE EFFECTIVENESS OF CULTURE MEDIUMS IN DETECTING CASES OF DEEP MYCOSES IN 162 PATIENTS

PATHOGENIC FUNGUS	SABOURAUD'S DEXTROSE AGAR	LITTMAN'S OXGALL AGAR
Sporotrichum schenkii	1	2
Coccidioides immitis	0	1
Blastomyces dermatitidis	0	2
Cryptococcus neoformans	1	1
Candida albicans	2	3
Total cases detected	4	9

the 9 cases of systemic mycoses discovered, exclusive of actinomycosis, 5 were not detected by Sabouraud's medium due to the profuse growth of secondary bacteria and fungi.

During the year's study all of the common pathogenic skin fungi were isolated from clinical material using Littman's oxgall agar. We were also able to isolate all of the fungi of the systemic mycoses, except the causative agents of histoplasmosis. The unsuitability of the medium for *Histoplasma capsulatum* has been described.[7] The majority of pathogenic fungi of both cutaneous and systemic mycoses grew on Littman's oxgall agar from clinical material in approximately the same time as noted for pure cultures. The unsuitability of the medium for the isolation of Actinomyces has already been noted.[6, 7]

DISCUSSION

Our favorable experience with Littman's oxgall agar and the new microslide for culture of fungi[8] indicate that routine culture diagnosis of pathogenic fungi is practical.

After a year's clinical trial of Littman's oxgall agar facts have been learned about the medium, the feasibility of performing routine fungus culture of clinical material and the training of technicians in medical mycology.

(1) The frequency of isolation of pathogenic fungi with Littman's oxgall agar and with it the specific diagnosis of fungus disease has been greatly increased; the technic has been greatly simplified.

(2) The ease of preparation of Littman's oxgall agar, especially with dehydrated medium, the stability of the poured medium and the advantages of room-temperature incubation are desirable features for clinical laboratories.

(3) The diagnosis of fungus disease by culture has proved to be less consuming of time and materials than that required for many bacterial diseases.

(4) A diagnosis by culture can be made early in a fungus disease before tissue reactions have developed. The favorable difference in prognosis in early detectable cases of coccidioidomycosis, blastomycosis and moniliasis over advanced cases is sufficient to warrant the effort of culture technics.

(5) We have found that the training of technicians in medical mycology requires a relatively short period of time.

The laboratory routine for fungus culture that is considered most suitable is described below.

Collection of specimens. Cleansing of the skin with acetone before scraping permits isolation of pathogenic fungi in pure culture when Littman's oxgall agar is employed. A fine powder of epidermal cells collected on a Bard-Parker blade is sufficient inoculum for the new medium.

Inoculation of medium. Deep plates of Littman's oxgall agar (25 ml.) are superior to slants because of the greater streaking surface provided. Skin and nail scrapings are spread over the plate with the flat side of the scraping knife. They may also be dropped on the surface of the agar during the scraping of the skin. Infected hairs are planted directly on the surface of the agar and should touch the surface at all points. Other specimens may be spread vigorously over the surface of the agar using sterile swabs. Streaking with a wire loop is not necessary. The contamination of plates by aerial fungi is not a serious problem because of the colonial restriction produced by the bile in the medium.

Conditions of incubation. This is done at room temperature and not at 37 C. which may reduce the number of positive cultures.[3, 6] A dust-free cabinet should be used, preferably of metal for easier disinfection. A large shallow pan of water should be installed to maintain favorable humidity. Plates maintained in this fashion will not dry excessively.

Examination of plates. Pathogenic skin fungi appear on Littman's oxgall agar by the fourth day of incubation and are well developed in seven days. The systemic pathogenic fungi appear on the medium in four to eight days. Candida species appear in 24 hours. It has been the practice to discard plates after 12 days' incubation if they fail to carry any suspicious fungus growth Although the appearance of most pathogenic fungi on the new medium was not very different from that seen on Sabouraud's agar, certain pathogenic fungi were very characteristic on it. The appearance of pathogenic fungi on both mediums is well illustrated in a recent "Atlas of Medical Mycology" (Moss and McQuown[9]).

It was observed that although sporulation of fungi was not inhibited on Littman's oxgall agar, fragmentation of the mycelium and dispersal of aerial spores by matured colonies was reduced; this observation along with the moist appear-

ance of fungi on the new medium, suggests a lessened danger to laboratory personnel in the handling of pathogenic fungi.

Identification of pathogenic species. Many species of fungi are easily recognized by their colonial appearance on the new medium and further identification is often unnecessary. This is especially true of saprophytic fungi.

Small sectors of the suspected pathogenic fungus colony on Littman's oxgall agar are cut away from the parent colony with a stout platinum wire flattened at the free end to the shape of a tiny spatula. Transplants are made simultaneously to: (1) Stock culture agar slant and (2) microslide culture[8] using potato dextrose agar[4] as a culture medium. The latter medium promotes the development of spores which comprise the main identifying characteristics of a fungus. Sabouraud's medium should not be used for microslide cultures since it favors the development of abundant mycelia which carry only few spores. Following a suitable period of incubation at room temperature, the Littman oxgall agar plate culture, the stock culture agar slant and the microslide cultures are examined and identification of the organism is made.

SUMMARY

A new selective agar culture medium for the primary isolation of pathogenic fungi known as Littman's oxgall agar, was applied to the culture diagnosis of cutaneous and systemic mycoses. Diagnoses of cutaneous mycoses were made 5 to 6 times more frequently with the new medium than with Sabouraud's dextrose agar, while systemic mycoses were detected twice as frequently. The laboratory routine for fungus culture considered most desirable is outlined. The high efficiency of the new medium in detecting pathogenic fungi from clinical material should make it useful to clinical and public health laboratories.

Acknowledgments. Miss Janis Smith and Miss D. M. Douglas furnished technical assistance. Dr. Emma Moss provided laboratory facilities and gave her whole-hearted cooperation for which we are most grateful.

REFERENCES

1. Bernhardt, E.: A simplified method for culturing fungi from the scalp. New England J. Med., **231:** 703–705, 1944.
2. Boeing, P. J., and Laffer, N. C.: A preliminary report on a selective medium for the isolation of pathogenic fungi. J. Bact., **54:** 90–91, 1947.
3. Howell, A.: The efficiency of methods for the isolation of *Histolpasma capsulatum*. Pub. Health Rep., **63:** 173–178, 1948.
4. Johnson, E. A.: An improved slide culture technique for the study and identification of pathogenic fungi. J. Bact., **51:** 689–694, 1946.
5. Leise, J. M., and James, L. H.: An alkaline medium and procedure for the selection of dermatophytes in the presence of saprophytic fungi. J. Lab. and Clin. Med., **30:** 119–131, 1945.
6. Littman, M. L.: A culture medium for the primary isolation of fungi. Science, **106:** 109–111, 1947.
7. Littman, M. L.: Growth of pathogenic fungi on a new culture medium. Am. J. Clin. Path., **18:** 409–420, 1948.
8. Littman, M. L.: A special microslide for culture of fungi. Am. J. Clin. Path., **19:** 278–283, 1949.
9. Moss, E. S., and McQuown, A. L.: An Atlas of Medical Mycology. Baltimore: The Williams and Wilkins Company, in press.
10. Steves, R. J., and Lynch, F. W.: Ringworm of the scalp; report of the present epidemic. J.A.M.A., **133:** 306–309, 1947.
11. Thompson, L.: Note on a selective medium for fungi. Proc. Staff Meet., Mayo Clin., **20:** 248–249, 1945.

A FROZEN SECTION TECHNIC*

JOHN B. HAZARD, M.D., AND GEORGE F. STEVENSON, M.D.

From the Cleveland Clinic and the Frank E. Bunts Educational Institute, Cleveland, Ohio

Many variations in the technic of preparing frozen sections have been devised but no one modification has proved universally satisfactory. The methods described in this paper, based on old and well-known procedures, obviate many of the difficulties inherent in rapid section technic, without increasing the complexity of the procedure.

MATERIALS

A. Equipment

In addition to a frozen section microtome, the following apparatus is required:

Heating unit, such as Fisher or Bunsen burner, electric plate or alcohol burner
1 Pyrex beaker (100 ml.)
Several glass rods (3 to 7 mm. in diameter)
1 large Stender dish (10 cm. in diameter and 5 cm. in height)
2 small Stender dishes with covers (5 cm. in diameter and 2.5 cm. in height)
1 wide-mouth glass jar (approximately 10 cm. in diameter and 10 cm. in height)
Microscope slides and coverslips

This equipment, with accessory instruments, is shown suitably arranged in Figure 1.

The glass rods and the microscope slides must be clean and free of film. This may be accomplished by soaking them in tincture of green soap, washing in running water, rinsing in 95 per cent alcohol and drying with a clean towel. Absolute alcohol is unsuitable for rinsing and unwashed gauze cannot be used for drying.

B. Fixative

An acetic acid-alcohol-formalin fixative is ideally suited to the method because it fixes rapidly at a low boiling point, increases the polychrome effect of the staining with toluidine blue, causes minimal tissue distortion, and retains the nuclear and other tissue detail. It is prepared according to the following composition:

Formalin 1:10, aqueous	70 ml.
Ethyl alcohol, 95 per cent	29 ml.
Glacial acetic acid	1 ml.

This fixative should be stored in a glass-stoppered bottle.

C. Stains

1. Modified toluidine blue. Mortar 1 Gm. of toluidine blue powder with 20 ml. of 95 per cent alcohol and add 80 ml. of distilled water and 1 ml. of glacial acetic

* Received for publication, June 7, 1949.

acid. Filter daily or before use into a small Stender dish. Toluidine blue thus prepared may be used for about two months.

2. Carbol-fuchsin. Dilute 1 volume of Verhoeff's carbol-fuchsin stock stain[1, 2] with 24 volumes of distilled water. Filter daily or before use into a small Stender dish. This stain should be effective indefinitely.

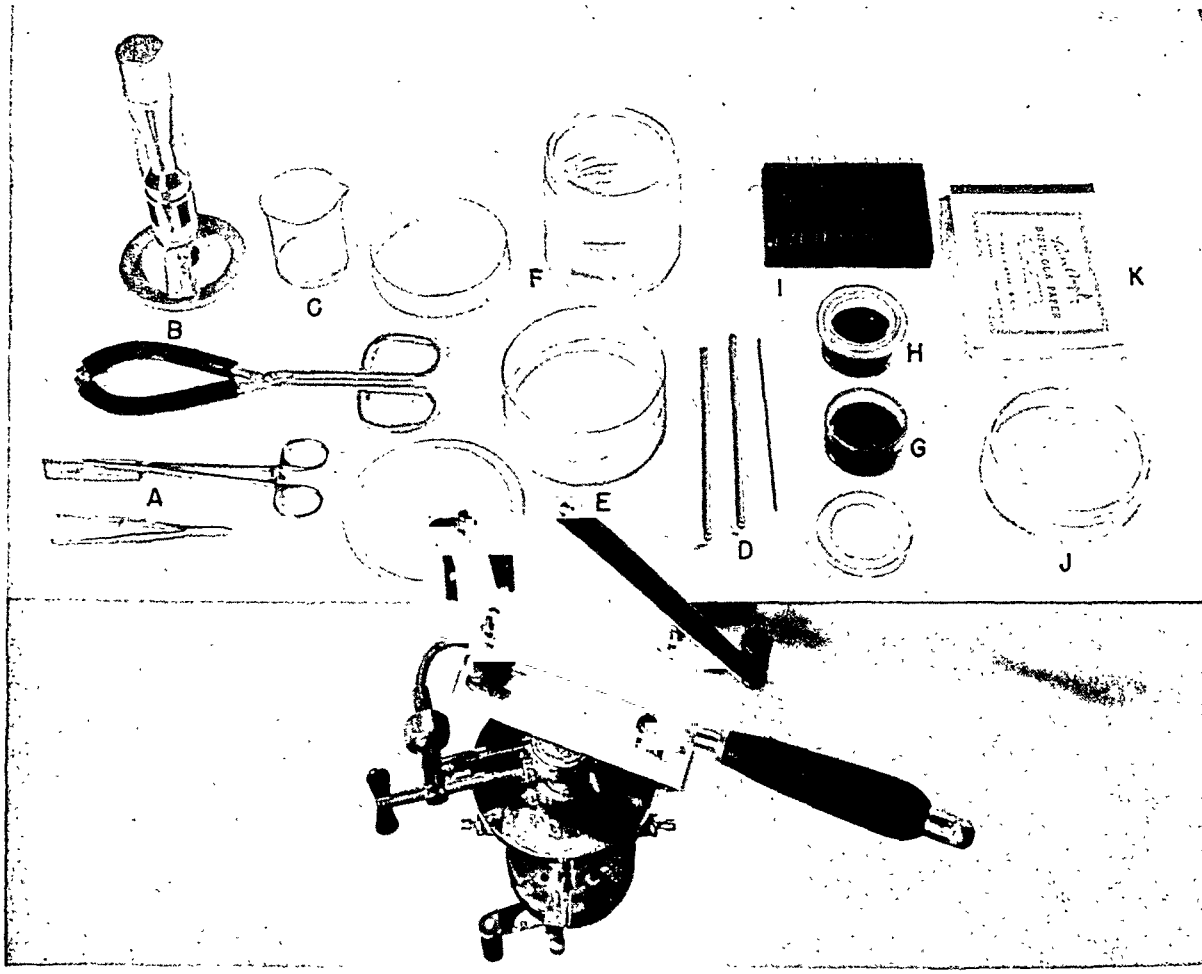


FIG. 1. Frozen section apparatus. A, Forceps and razor for cutting blocks; B, Fisher burner; C, beaker, beaker cover (Petri dish), and beaker holder; D, glass rods; E, large Stender dish; F, glass jar; G and H, small Stender dishes containing stains; I, microscope slide holder with slides; J, coverslips; K, blotting paper.

TECHNIC

Fixation

A block of tissue, removed from a fresh specimen, is placed in 10 times its volume of fixative in a beaker and heated just to the boiling point over a Fisher burner or another suitable heater. If the vapor becomes ignited when using an open flame, the beaker should be covered briefly with an inverted Petri dish. Refixation, after trimming the blocks to a thickness of 3 mm., may be desirable

in making frozen sections of soft tissues, such as lymph nodes or thyroid tissue, especially when the capsule of a tumor is to be examined, and of other tissues which are grossly sectioned with difficulty. To insure a more or less level surface, particularly in the case of large or thin blocks, the specimen should be placed on a small dry piece of stiff, fine-mesh filter paper before dropping it into the fixative. Formalin is satisfactory as a fixative but is inferior to acetic acid-alcohol-formalin. The result with Zenker fixation is unsatisfactory because of difficulties in immediate sectioning.

Sectioning

The fixed tissue is frozen and cut at a thickness of 10 to 15 microns, using a plano-concave microtome knife and taking care that its heel does not strike the tissue. The knife is moistened with water at the cutting point and each section, as it is cut, is wiped off the blade by a finger and transferred to a jar filled with distilled water or physiologic saline. Troublesome bubbles may be encountered if tap water is used.

Staining

A clean glass rod is used to transfer the tissue to the staining jar. Two methods of staining may be employed: (1) carbol-fuchsin and toluidine blue or (2) toluidine blue alone. Staining with carbol-fuchsin and toluidine blue is used as a routine technic in the following manner.

Carbol-fuchsin and toluidine blue:

Stain in dilute carbol-fuchsin solution, 3 seconds.
Rinse in distilled water.
Stain in toluidine blue solution, 6 seconds.
Rinse in distilled water.

This stain produces coloring similar to that of the familiar hematoxylin and eosin preparations. The polychrome effect is given by toluidine blue when used alone, as follows:

Toluidine blue:

Stain in toluidine blue solution, 6 seconds.
Rinse in distilled water.

The sections are handled in the same manner for both staining methods. While still on the rod, the section is immersed in the stain for the time designated. Care must be taken not to lose the section in the stain. This may be avoided by submerging the tissue partially on the end of the glass rod and rotating the latter so that opposite halves of the section are passed alternately into the stain (Fig. 2 A). Then, holding the rod horizontally, the section is rolled onto the surface of distilled water in a deep glass jar (Fig. 2 B). This is accomplished very easily if the water fills the jar just to the brim. Surface tension prevents wrinkling or disintegration of the section. The section is rinsed by lifting it on a glass rod and then refloating it on the water repeatedly until excess stain is removed. In the transfer from water to stain it is desirable to touch the rod to filter paper to prevent dilution of the stain.

Mounting

A clean glass slide is lowered perpendicularly into the water, brought to the edge of the section, and lifted vertically (Fig. 2 C). Thus, supported by surface tension, the stained section is readily transferred to the slide. Generally the section is easily mounted intact; if necessary, however, it may be partly refloated and mounted as desired.

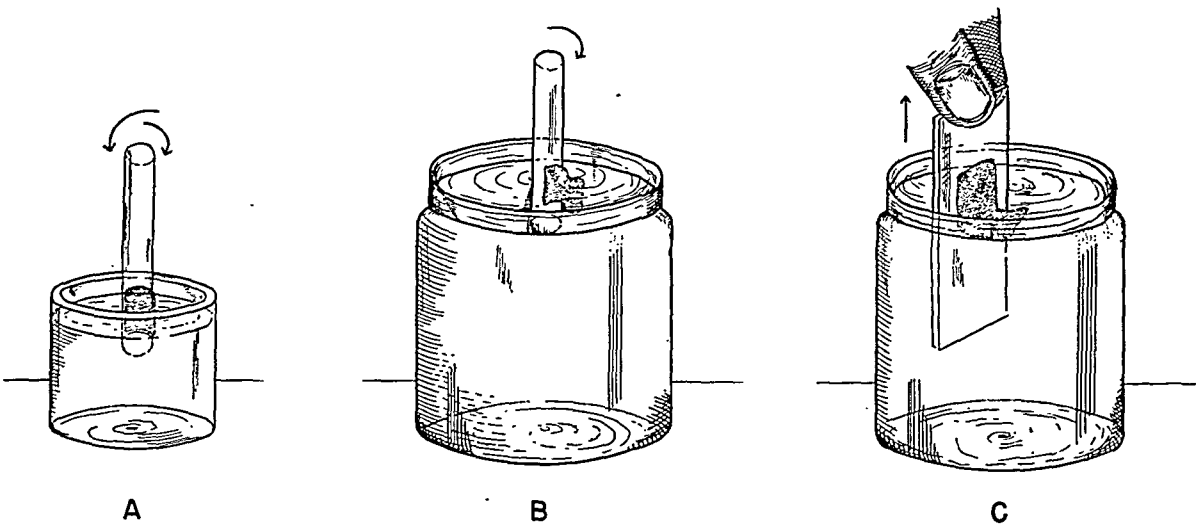


FIG. 2. Diagram illustrating steps in frozen section technic. A, Staining: opposite halves of a section, held on the end of a glass rod, are alternately rotated into the stain. B, Rinsing: with the glass rod held almost horizontally, the section is unrolled and allowed to float onto the surface of the distilled water. C, Mounting: a slide, held at the edge of the floating section, is lifted vertically, carrying with it the intact section.

The method of mounting, in routine use, is as follows:

1. Mount section on slide as above.
2. Blot gently with smooth filter paper.
3. Cover immediately with a drop of 30 per cent sucrose or cane sugar solution.
4. Place coverslip in position.
5. Place slide between sheets of smooth filter paper and blot lightly.

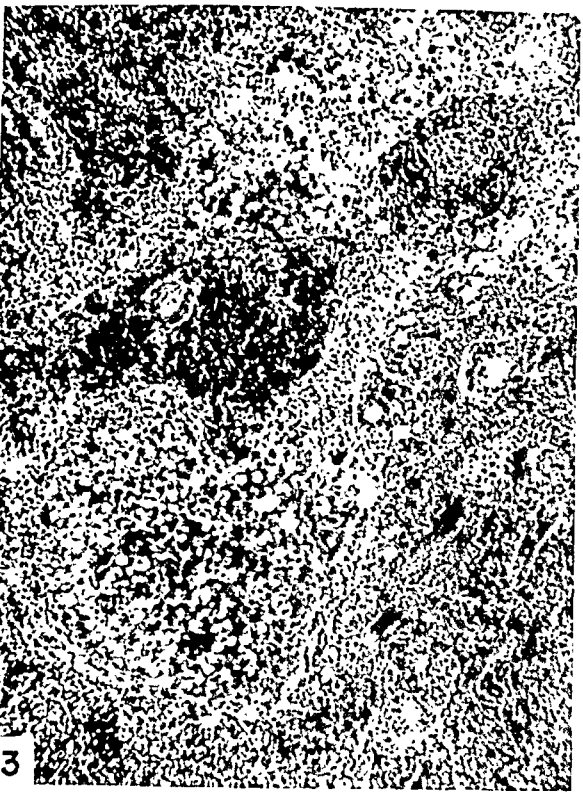
Sections mounted in this manner remain free of bubbles and will not show evidence

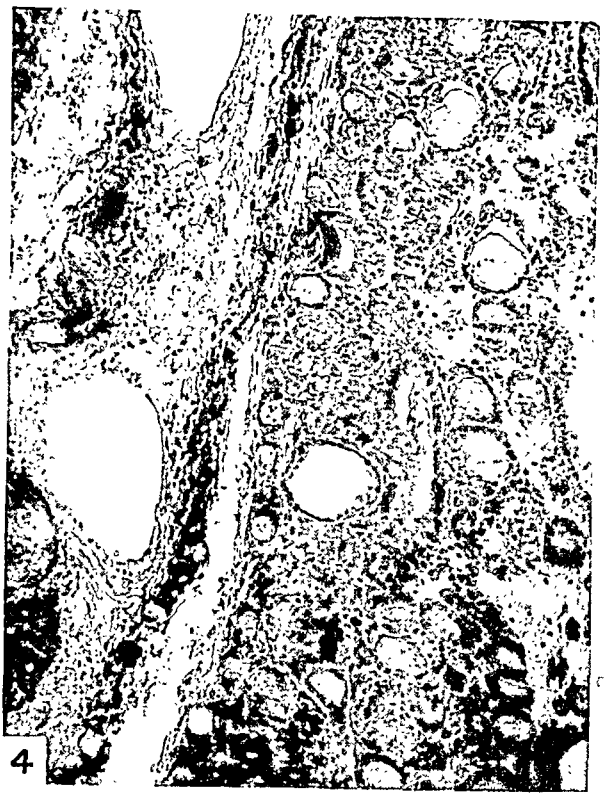
FIG. 3. Secondary colloid carcinoma of lymph node. Tissue block (1.5 x 1 x 0.5 cm.), was fixed in acetic acid-alcohol-formalin with heating, trimmed to 2 mm. thickness, and refixed; sections were cut at 15 microns, stained in toluidine blue for 6 seconds and mounted in water. × 70.

FIG. 4. Adenoma of thyroid. Tissue block (3 x 2 x 1.5 cm.), was fixed in acetic acid-alcohol-formalin with heating, trimmed to 3 mm. thickness, and refixed; sections were cut at 12 microns, stained in carbol fuchsin for 3 seconds, rinsed, counterstained with toluidine blue for 6 seconds, and mounted in cane sugar solution. × 70.

FIG. 5. Chronic gastritis. Tissue block (2 x 2 x 0.7 cm.) of gastric wall, was fixed in acetic-acid-alcohol-formalin with heating, trimmed to 2 mm. thickness, and refixed; sections were cut at 12 microns, stained in toluidine blue for 6 seconds and mounted in water. × 70.

FIG. 6. Astrocytoma of brain. Fragment of tissue (3 x 2 x 2 mm.) was fixed in acetic acid-alcohol-formalin with heating; sections were cut at 10 microns, stained in carbol fuchsin for 3 seconds, rinsed, counterstained in toluidine blue for 6 seconds and mounted in cane sugar solution. × 250.









FIGS. 3–6

of drying for several days, so that they may be retained while awaiting permanent sections. They may be preserved longer by coating the coverslip margins with flexible collodion. If the tissue is mucinous or otherwise unsuited for direct blotting, steps 2 and 3 in the above procedure should be omitted. Sections thus prepared are suitable for study for approximately an hour. They may be preserved in good condition for several days, however, by rimming the coverslip with "Permount" or a similar quick-drying mounting agent, and storing in a refrigerator.

DISCUSSION

The primary requisites for a frozen section technic, simplicity, speed, and uniformity of results, are fulfilled by this method or slight variations of it. Its simplicity permits mastery in a short time, often after only a few attempts. The method is applicable to all types of tissues with uniform success; mechanical imperfections, such as shredding and wrinkling, are reduced to a minimum, even with unusually wide blocks of tissue.

The method of combined staining with carbol-fuchsin and toluidine blue, and mounting in 30 per cent sucrose or cane sugar, after flattening the section by blotting, is used as the routine technic because it possesses several advantages. The purple nuclei and red tissue elements of such sections closely resemble those seen in the commonly-used hematoxylin and eosin preparations. An additional advantage is found in the identification of intercellular fibrillary substances, such as glial fibers, which stand out distinctly; this is especially valuable in examining tumors of the glioma group where the presence of fibers is of diagnostic importance. The mounting technic produces flat sections with distinct cellular components that may be preserved for several days or longer.

Toluidine blue, when used alone, has the property of polychrome staining, making certain types of cells or substances readily identifiable. Generally, nuclear substance stains deep purple to black whereas cytoplasm varies somewhat in staining reaction. The following tissue elements exhibit constant staining properties:

elastic tissue, refractile green-blue
collagenous tissue, pale red with a blue cast, or unstained
smooth muscle, pale blue
epithelium, pale blue-purple
polymorphonuclear neutrophils, pale green or unstained
plasma cells, dark lavender
mast cells, granular or diffuse red-purple
myelin, light to dark red
fibrin, pale yellow-green or unstained
mucus (epithelial), red, purple, or unstained

The elastic tissue of the elastic lamina of arteries has a distinctive appearance in that it is seen as a refractile green layer surrounded by red media; the red color is due to the deep red-staining matrix between smooth muscle cells. Variations in color also enable easy distinction between the white matter (light pink or red,

due to myelin) and the gray matter (darker pinkish blue) of the central nervous system. The demonstration of myelin is enhanced by the use of polarized light in which it appears brilliant pinkish red.

In addition to these methods of staining, sections prepared in the prescribed manner are suitable also for fat stains; Zenker-fixed tissues also may be adapted for fat stains.

Regardless of the variation of the staining method employed, the staining characteristics of the sections and the mechanical facility of the technic combine to give preparations a value that rivals that of the permanent method. In fact, in some circumstances, the specific nature of the staining reaction provides diagnostic features which are not seen in paraffin sections. In addition, there is the advantage of speed as well as the absence of cell distortion so common in dehydrated frozen section preparations.

SUMMARY

1. A simple standardized frozen section technic, embodying rapid fixation, differential staining and surface-tension mounting, is presented.

2. The method offers the advantages of technical facility, uniformity of results, and certain specific staining characteristics.

Acknowledgment. We wish to express our appreciation to Miss Alice Young for her helpful assistance in the development of the technic.

REFERENCES

1. LILLIE, R. D.: *Histopathologic Technic.* Philadelphia: The Blakiston Company, p. 215, 1948.
2. MALLORY, F. B.: Pathological Technique. Philadelphia: W. B. Saunders Company, p. 88, 1938.

USE OF MAXTED'S STREPTOMYCES FILTRATE FOR RAPIDLY EXTRACTING GROUP CARBOHYDRATES FROM HEMOLYTIC STREPTOCOCCI*

DOROTHY LONGFELLOW, B.S., SYLVIA G. CARY, B.S., AND L. R. KUHN, PH.D. (LT. COL., MSC)

From the Department of Bacteriology, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C.

A novel method of extracting group carbohydrates from hemolytic streptococci by using a proteolytic filtrate from a strain of *Streptomyces albus* was recorded recently by Maxted.[5] The description indicated that this method was much simpler and less time-consuming than the Lancefield acid-extraction[4] or Fuller formamide-extraction[3] technics. If successful, it would enable more small diagnostic laboratories to differentiate group A hemolytic streptococci, which are most frequently associated with epidemics, and to recognize other groups which occur in many non-epidemic infections.[1, 2] Accordingly, a comparison of the utility and specificity of the three methods was made employing all available strains of streptococci and grouping serums.

METHODS

Maxted's directions were closely followed.[5] In summary, a proteolytic substance is obtained from a culture of *Streptomyces albus* grown on a soft agar medium containing Fildes' extract. This substance, presumably an enzyme, is contained in the material expressed from the medium by freezing and thawing; it is then filtered and the pH is adjusted. In use, a loopful of streptococci from a blood agar plate is dissolved in 0.25 ml. of the filtrate. Lysis requires approximately 30 minutes. The resultant clear solution is used in the Swift, Wilson, Lancefield prodecure for grouping and typing streptococci by precipitation in capillary pipets.[6] The proteolytic filtrate is quite stable and can be made easily in large quantity.†

The Lancefield and Fuller extracts were prepared according to directions given in references cited previously.[3, 4] It was found during the course of the study that poor results which had been obtained previously with Lancefield extracts were due to a modification employing buffered phenol red instead of an aqueous solution. In the case of the Fuller extract, unless used immediately, readjustment of the pH is required because it becomes too alkaline on standing.

EXPERIMENTAL

In order to compare the Maxted extraction method with the Lancefield and Fuller technics, extracts of streptococci of known groups were prepared accord-

* Received for publication, May 5, 1949.

† Studies of stability at temperatures encountered in shipping are being made. If indicated, it could then be distributed from this or other central laboratories. Detailed mimeographed instructions for preparing the filtrate, with the modifications used at this laboratory may be obtained from the Department of Bacteriology, AMDR and GS, Army Medical Center, Washington 12, D. C.

ing to each method, and were set up against standard serums for all groups in accordance with the Lancefield precipitin test.

Using 9 known Group A strains, positive and specific Group A reactions were obtained with Maxted and Lancefield extracts from all 9. Fuller extracts from 2 of these strains, however, failed to react with Group A serum.

Using 2 stains each of known Groups B, C, E, F and G, positive and specific reactions were obtained with extracts prepared in accordance with all three procedures.

Maxted noted that streptococci which do not produce a soluble hemolysin also are not readily dissolved by the proteolytic filtrate. In such cases, saline suspensions of the colonies are immersed in boiling water for three to four minutes, then cooled before the proteolytic filtrate is added. The reason for this correlation of susceptibility to lysis and production of hemolysin is not known. Maxted stated

TABLE 1

CLASSIFICATION OF HEMOLYTIC STREPTOCOCCI BY USE OF MAXTED'S AND OTHER EXTRACTS

NO. OF STRAINS	GROUPING OF ORGANISMS AFTER TREATMENT BY DESIGNATED EXTRACT		
	Maxted's Extract	Lancefield's Extract	Fuller's Extract
1	Group A	0	0
1	Group A	Group A	Group A
1	Group B	Group B	Group B
1	Weak group B (strong with modified method)	Group B	Group B
1	Group C	Group C	Group C
6	Group D	Group D	Group D
1	Weak group D (strong with modified method)	0	Weak group D
1	0 (group D with modified method)	Group D	Group D

(personal communication) that in epidemiologic studies he eliminates organisms which do not produce soluble hemolysin, thereby saving considerable time and serum. Of course, some nondescript streptococci lyse readily. In our studies, 2 stock strains of non-hemolytic Group D streptococci could not be grouped by the original Maxted method, even when dissolved after heating, although Group D reactions were obtained with the Lancefield and Fuller technics. However, when the centrifugate from 5 ml. of a 24-hour broth culture, instead of a loopful of organisms, was incubated at 50 C. with filtrate, an extract was obtained which gave strong Group D reactions. It is our practice to use this modification in attempting to group all questionable or non-hemolytic streptococci.

In addition to the above study, 13 unknown strains of freshly isolated streptococci were studied by the 3 methods of extraction. The results are given in Table 1.

In a third study, various lots of available standard grouping serums were tested with extracts of 2 strains each of Groups A, B, C, D, E, F and one of Group G streptococci, all prepared by the Maxted, Lancefield and Fuller methods.

Except for 3 lots, the potency and specificity of the serums were adequate for diagnostic purposes when used with homologous extract prepared by any of the 3 methods. The 3 poor lots were equally inadequate with all types of extracts.

SUMMARY

From these limited preliminary studies, it appears that, with indicated modifications, the Maxted method is a simple and practical technic for extracting group carbohydrates. It avoids time-consuming centrifugations and adjustments of pH, and is stable. Agar growth of strains producing soluble hemolysin can be used directly without waiting for growth in broth. Proteolytic filtrate can be furnished to small installations by a central laboratory and thus the number of installations which can perform streptococcus grouping could be increased, with very little additional expenditure of their time. In the few laboratories where grouping *and* typing of Group A hemolytic streptococci are performed, the Lancefield extract should first be used since it contains carbohydrate and protein fractions for both grouping and typing. In such laboratories the Maxted or Fuller methods would be employed when weak or cross reactions are obtained.

REFERENCES

1. Commission on Acute Respiratory Diseases: The role of Lancefield groups of beta-hemolytic streptococci in respiratory infections. New England J. Med., **236:** 157–166, 1947.
2. Foley, G. E.: Further observations on the occurrence of streptococci of groups other than A in human infections. New England J. Med., **237:** 809–811, 1947.
3. Fuller, A. T.: The formamide method for the extraction of polysaccharides from hemolytic streptococci. Brit. J. Exper. Path., **19:** 130–139, 1938.
4. Lancefield, R. C.: Serological differentiation of human and other groups of hemolytic streptococci. J. Exper. Med., **57:** 571–595, 1933.
5. Maxted, W. R.: Preparation of streptococcal extracts for Lancefield grouping. Lancet, 255–256, 1948.
6. Swift, H. F., Wilson, A. T., and Lancefield, R. C.: Typing group A hemolytic streptococci by M precipitin reactions in capillary pipettes. J. Exper. Med., **78:** 127–133, 1943.

USE OF KÖHLER ILLUMINATION FOR RETICULOCYTE COUNTS*

JOHN D. KENNEDY, M.D.

From the Regional Laboratory Service, Central Hospital, Galway, Ireland

The use of a restrictive eyepiece is generally advised by standard textbooks in clinical pathology for the counting of reticulocytes by the "wet" or "dry" method. Binocular microscopes are now being used more generally and these do not lend themselves to the use of restrictive eyepieces. Köhler illumination, imperative for microphotography, gives wonderfully good definition of the reticulin network and its particles within the reticulocyte. The method has the advantage that once the back lens of the 4 mm. objective has been filled with light, the periphery of the field can be darkened to the desired extent by further closure of the iris diaphragm. Counting can be confined to the central portion of the field, the edge of which is sharply defined by the shadow of the iris diaphragm. This method gives all the advantages of a restrictive eyepiece to the binocular microscope. Excellent definition is obtained. The lamp must, of course, be provided with a condenser and an iris diaphragm.

Köhler illumination. Focus the condenser of the lamp so that the image of the bulb filament is seen in sharp focus on the closed iris diaphragm. The edge of the image should have a bluish tinge. Open the iris diaphragm. Focus the microscope using the 4 mm. objective on the preparation. Narrow the aperture of the lamp diaphragm. Focus the microscope condenser until the diaphragm of the lamp is in focus with the specimen. The lamp diaphragm is closed until only the field is illuminated. When the eyepieces are removed it should be possible to see the image of the lamp fill the back lens of the objective. Once this has been achieved, a suitable filter may be inserted to cut down excessive brightness. The size of the field may then be restricted to the extent desired by closing the iris diaphragm of the lamp and then closing the iris of the microscope until there is a slight diminution in the brightness of the portion of the field illuminated.

* Received for publication, June 6, 1949.

NOMOGRAPH FOR RAPID CALCULATION OF ICTERUS INDEX WHEN DETERMINED BY COLORIMETER*

ROBERT J. HOSLEY, B.A.†

Stitt *et al.*[2] claim that the use of the colorimeter in icterus index determination implies a degree of precision which the test does not possess. In higher serum dilutions, however, the element of human error introduced by the use of a simple comparator tube, rather than the colorimeter, may be as great as 15 to 20 units. Hence, the Meulengracht method, as modified by Bernhard and Maue[3] for the Duboscq colorimeter, is perhaps the most widely used in our clinical laboratories today.

The use of charts for the rapid calculation of plasma proteins, hemoglobin, and hematocrit, such as those introduced by Phillips *et al.*,[1] considerably lessens the work of many laboratories. This was the case in our laboratory. During the summer months of 1945, the volume of routine icterus determinations became so great in our laboratory that one technician spent most of the afternoon on this work alone. With the thought in mind of saving as much time as possible without, of course, sacrificing accuracy, the author devised a nomograph to facilitate calculation (Fig. 1).

One example of the operation of this nomograph will suffice. The "standard" cup of the colorimeter is filled with the dichromate standard solution and set at 20. One ml. of the serum to be tested is diluted with saline to the approximate color intensity of the standard solution, placed in the "unknown" cup of the colorimeter, and the conventional readings are taken. If, for example, 46 ml. of saline has been used to dilute the serum, and the vernier scale for the unknown reads 23, a taut string or a straight edge placed across the nomograph from 46 on the saline scale (S) to 23 on the colorimeter scale (Ru) will give a reading of 40, on the center scale (I.I.), as the icterus index.

SUMMARY

A nomograph for the rapid calculation of the icterus index, using the Bernhard and Maue colorimetric method, has been presented. The chart is based on a colorimeter standard set at 20, and a serum quantity of 1 ml. The saline dilution scale was found to be adequate for all cases of clinical and subclinical jaundice encountered in our work.

* Received for publication, May 2, 1949.

† Address, Todd Union, University of Rochester, Rochester 3, New York.

REFERENCES

1. Phillips, R. A., Van Slyke, D. D., Dale, V. P., Emerson, K., Hamilton, P. B., and Archibald, R. M.: Copper sulfate method for measuring specific gravities of whole blood and plasma. Rockefeller Institute for Medical Research, pp. 47, 49, 1942.
2. Stitt, E. R., Clough, P. W., and Clough, M. C.: Practical Bacteriology, Haematology, and Animal Parasitology. Ed. 9. Philadelphia: The Blakiston Company, p. 765, 1943.
3. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, Ed. 11 Philadelphia: W. B. Saunders Company, p. 424, 1948.

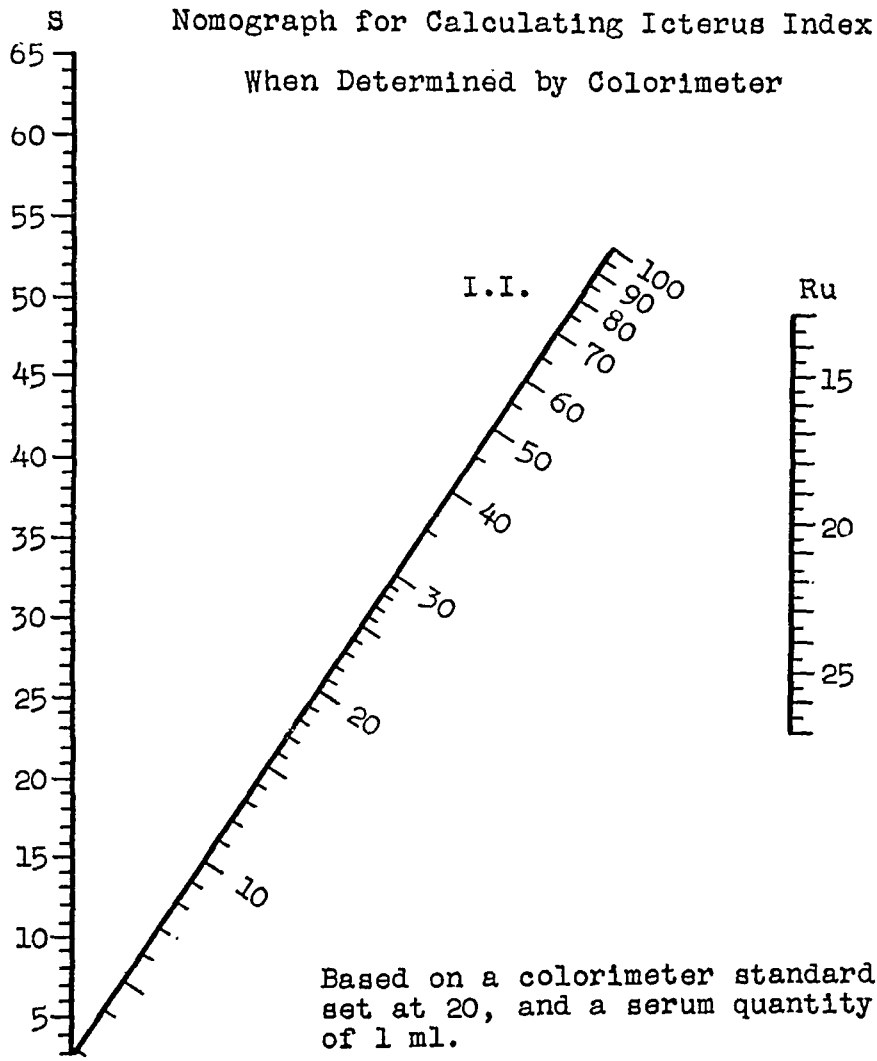

Nomograph for Calculating Icterus Index
When Determined by Colorimeter
S
65
60
55
50
45
40
35
30
25
20
15
10
5
I.I.
100
90
80
70
60
50
40
30
20
10
Ru
15
20
25
Based on a colorimeter standard set at 20, and a serum quantity of 1 ml.

FIG. 1

DETERMINATION OF PARA-AMINOSALICYLIC ACID BLOOD LEVELS*

BENJAMIN J. WILSON, M.S., AND BRUCE W. ARMSTRONG, M.D.

From the Clinical Laboratory of the Hopemont Sanitarium, Hopemont, West Virginia

In 1946 Lehmann[10] announced that para-aminosalicylic acid (PAS) produced a 50 to 75 per cent inhibition of growth of an attenutated bovine tubercle bacillus *in vitro*. This has been confirmed by other investigators.[12, 13, 16] Youmans and associates,[17, 18] Feldman *et al.*,[4, 5] and McClosky *et al.*,[11] found a definite *in vivo* effect of the drug on experimental tuberculosis in white mice, guinea pigs, and rabbits.

In studies on human tuberculosis, Lehmann,[10] Erdei,[3] and Dempsey and Logg[2] reported results which indicated a tuberculostatic effect, although the observation periods were too short for critical appraisal. Karlson *et al.*[8] treated tuberculous patients with PAS in conjunction with streptomycin and reported that of 14 strains of tubercle bacilli isolated from as many patients, only one was resistant *in vitro* to streptomycin after three months of therapy with streptomycin; 5 patients had been treated with streptomycin and PAS, one with streptomycin and Promin, and 8 with streptomycin, PAS and Promin. Graessle and Pietrowski[6] investigated the *in vitro* effect of PAS on the emergence of streptomycin-resistant cultures of the tubercle bacillus and found that concentrations of 0.5 microgram of PAS to 1 unit of streptomycin prevented or greatly retarded the development of streptomycin resistance.

Way *et al.*[14] reported blood levels of 9 persons who were given doses of 2, 2.5, and 4 grams of PAS. McClosky and co-workers[11] stated that the diazotization procedure which was used by Way gave erratic results. This was to be suspected from the blood level curve published by Way *et al.*, since the levels obtained with 2-gram doses were occasionally greater than when 4 Gm. were given. This curve indicated that an oral dose of 2 to 4 Gm. of PAS every four to six hours would maintain bacteriostatic blood levels throughout the day and night. Way and associates, on the basis of their data on urinary excretion of PAS, stated that the drug should be given every four hours.

We have investigated the possibility of increasing the dosage interval to eight hours while maintaining *in vitro* bacteriostatic blood concentrations constantly. Such a spacing of dosage would be convenient since the drug often produces nausea and vomiting shortly after administration.

METHOD

The blood levels of PAS on 11 tuberculous and 7 non-tuberculous persons were checked by a modification of the method of Keller[9] which measures the free phenol group. Comparative tests to determine free amine-group concentrations were made on 6 of the 18 persons, according to the method of Bratton.[1]

* Received for publication, June 27, 1949.

On the day before therapy, the recipients were allowed no salicylates. Samples of fasting-blood were collected and readings were obtained of the ferric nitrate reagent and nitric acid control from the Leitz photoelectric colorimeter, using a 5250 Å color filter. These readings were recorded individually for each person taking the drug. Five Gm. of PAS was suspended in water and given orally to each person to be tested. The blood levels were checked at intervals of 1, 2, 4, 6, 8, and 12 hours.

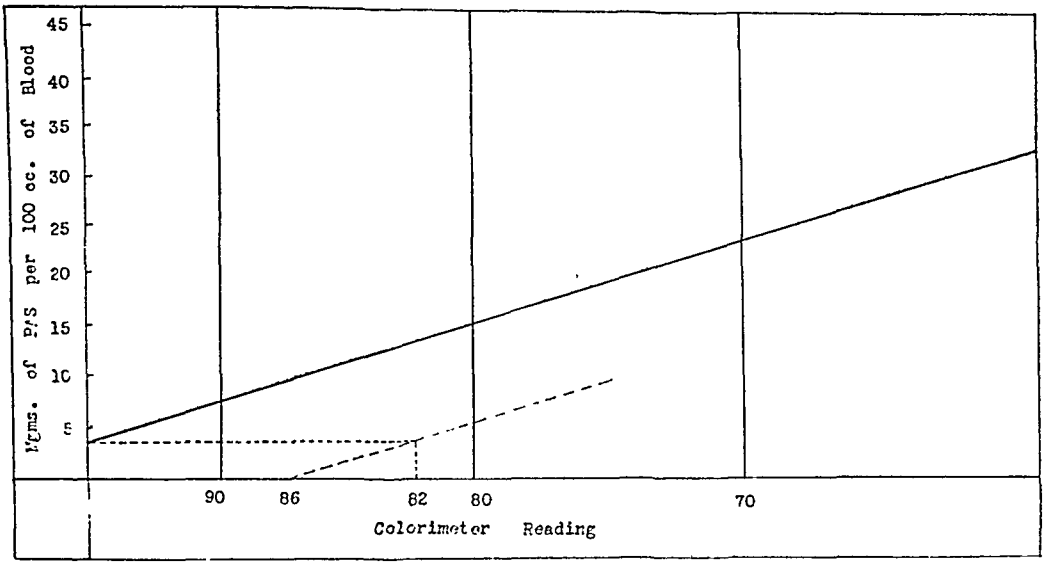


FIG. 1. Solid line represents curve derived from colorimeter readings on standard aqueous solutions containing known amounts of para-aminosalicylic acid per 100 ml. The dotted line illustrates the example cited in the test.

The values of PAS in mg. per 100 ml. blood were derived from a line drawn from readings on standard solutions on the logarithmic graph, as illustrated in the following example (also see Fig. 1):

TIME	Colorimeter Readings	
	Ferric Nitrate Reagent and Serum	HNO_3 Serum Control
Fasting	86	92
1 hour after administration of 5 Gm. PAS	70	80

The fasting acid-serum value, 92, minus the 1-hour acid-serum value, 80, equals 12; the 1-hour ferric nitrate-serum value, 70, plus 12 equals 82 or the corrected 1-hour ferric nitrate reading. The value of the corrected 1-hour reading was then determined from the line which began at 86 and was drawn parallel to the standard line (See Fig. 1).

Sufficient blood was collected from 6 persons to run determinations on the

serum, oxalated blood, plasma, and packed cells. The serums were checked by the ferric nitrate method of Keller,[9] as described above, while the whole blood, plasma, and red cells were tested by the diazo method of Bratton.[1]

Twenty-four hour urinary excretion of PAS was determined, as in the case of serum, using the ferric nitrate reagent. No zero reading was necessary, since the preliminary filtration and fifty-fold dilution of urine containing no PAS gave a galvanometer reading of 99–100. Thus, PAS concentrations were determined from the original curve plotted on the logarithmic graph for the standard solutions. The volume of urine was measured and the total output of PAS was calculated.

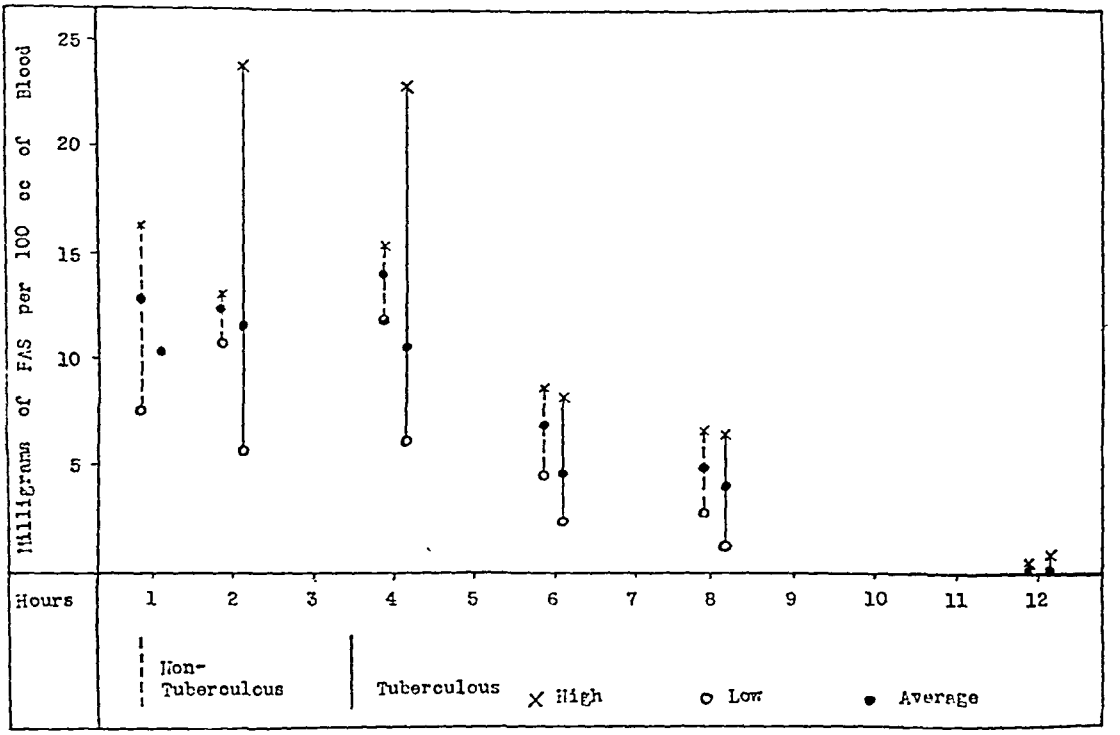


FIG. 2. Para-aminosalicylic acid blood levels after 5 Gm. oral dose

RESULTS

Figure 2 shows the results of the blood level determinations. Although the individual concentrations varied, it was noted that unlike rheumatic fever patients, who demonstrated higher salicylate levels than normal subjects,[7] tuberculous patients had average PAS levels that were essentially the same as those of the normal persons tested. Fairly high concentrations were reached in one to two hours and decreased gradually to a zero value within twelve hours.

In the 6 persons whose plasma PAS levels were checked by both the ferric nitrate and the diazo methods, no general correlation was found. Although the values obtained by the two methods sometimes paralleled each other, they often differed by as much as 15 to 20 per cent.

In the cases where the free amine group concentration was determined by

the diazo reaction on whole blood, plasma, and packed erythrocytes, the two fractions showed essentially the same values as whole blood. Similar results were tabulated by Way and associates[14] in some of the subjects they tested. However, there was a preponderance of evidence in their over-all results to indicate that a greater percentage of the drug was present in the plasma than in the erythrocytes.

The total urinary excretion of the drug during the 24-hour period following administration ranged from 49 per cent to 86 per cent of the amount given. The average for the tuberculous group was 66.5 per cent and for the normal persons, was 67.7 per cent.

DISCUSSION

Lehmann,[10] Sievers,[12] and Youmans[16] agree that the *in vitro* tuberculostatic concentration of PAS is in the vicinity of 0.019 to 0.156 mg. per 100 ml. of liquid medium. These findings have been confirmed by us on solid medium.[15]

The observations of Way *et al.*[14] indicate that bacteriostatic levels of PAS as determined by the diazo reaction are maintained for at least six hours on both 2 and 4-gram doses. However, knowledge available at present would seem to indicate that PAS blood levels are maintained in the *in vitro* bacteriostatic range when a dose of 5 Gm. of the drug is given at eight hour intervals.

SUMMARY AND CONCLUSIONS

The blood level of para-aminosalicylic acid was determined in 11 tuberculous patients and in 7 normal persons. A modification was employed of the method described by Keller, which determines the phenol group concentration. The levels determined were compared with those obtained by Way *et al.*, and by us, using a diazo method.

The urinary output of the drug was determined by a modification of the method of Keller. The 24-hour excretion averaged 67 per cent of the amount ingested.

Following a 5-gram dose of para-aminosalicylic acid, the blood maintains concentrations for eight hours which have been found to be tuberculostatic *in vitro*.

REFERENCES

1. Bratton, A. C., and Marshall, E. K., Jr.: A new coupling component for sulfanilamide determination. J. Biol. Chem., **128:** 537–550, 1939.
2. Dempsey, T. G., and Logg, M. H.: Para-aminosalicylic acid in tuberculosis. Lancet, **2:** 871, 1947.
3. Erdei, A. and Snell, W. E.: Pulmonary tuberculosis treated with p-aminosalicylic acid. Lancet, **1:** 791–793, 1948.
4. Feldman, W. H., Karlson, A. G., and Hinshaw, H. C.: Para-aminosalicylic acid in experimental tuberculosis in guinea pigs. Proc. Staff Meet., Mayo Clinic, **22:** 473–479, 1947.
5. Feldman, W. H., *et al.*: Parenteral administration of p-aminosalicylic acid (PAS) in experimental tuberculosis. Proc. Staff Meet., Mayo Clinic, **24:** 220–224, 1949.
6. Graessle, O. E., and Pietrowski, J. J.: The *in vitro* effect of para-aminosalicylic acid (PAS) in preventing acquired resistance to streptomycin by *mycobacterium tuberculosis*. J. Bact., **57:** 459–464, 1949.
7. Huntington, R. W., Jr., *et al.*: Studies in rheumatic fever; absorption of salicylates. Ann. Int. Med., **24:** 1029–1038, 1946.

8. KARLSON, A. G., *et al.*: The effect of combined therapy with streptomycin, para-aminosalicylic acid and promin on the emergence of streptomycin resistant strains of tubercle bacilli; a preliminary report. Proc. Staff Meet., Mayo Clinic, **24**: 85–88, 1949.
9. KELLER, W. J., JR.: A rapid method for the determination of salicylates in serum or plasma. Am. J. Clin. Path., **17**: 415–417, 1947.
10. LEHMANN, J.: Para-aminosalicylic acid in the treatment of tuberculosis. Lancet, **1**: 15–16, 1946.
11. MCCLOSKY, W. T., SMITH, M. I., AND FRIAS, J. E. G.: The action of p-aminosalicylic acid (PAS) in experimental tuberculosis. J. Pharmacol. and Exper. Therap., **92**: 447–453, 1948.
12. SIEVERS, O.: Experimental research on p-aminosalicylic acid and various species of bacteria. Svenska läk. Tidnong, **43**: 2041, 1946.
13. VENNESLAND, K., EBERT, R. H., AND BLOCH, R. G.: *In vitro* effect of streptomycin and para-aminosalicylic acid (PAS) on the growth of tubercle bacilli. Proc. Soc. Exper. Biol. and Med., **68**: 250–255, 1948.
14. WAY, E. L., *et al.*: The absorption, distribution, excretion and fate of para-aminosalicylic acid. J. Pharmacol. and Exper. Therap., **93**: 368–382, 1948.
15. WILSON, B. J., AND ARMSTRONG, B. W.: Unpublished observations.
16. YOUMANS, G. P.: The effect of para-aminosalicylic acid *in vitro* and *in vivo* on virulent human type tubercle bacilli. Quart. Bull. Northwestern Univ. M. School, **20**: 420, 1946.
17. YOUMANS, G. P., RALEIGH, G. W., AND YOUMANS, A. S.: The tuberculostatic action of para-aminosalicylic acid. J. Bact., **54**: 409–416, 1947.
18. YOUMANS, G. P., YOUMANS, A. S., AND OSBORNE, R. P.: The combined effect of streptomycin and para-aminosalicylic acid on experimental tuberculosis in mice. Lancet, **67**: 403–404, 1947.

USE OF SPONGE IN OBTAINING TISSUE FROM STOMACH FOR BIOPSY*

SIDNEY A. GLADSTONE, M.D.

From the Department of Pathology, New York Polyclinic Medical School and Hospital, New York, New York

The use of sponge in collecting material for biopsy in suspected cancer of accessible organs such as the cervix uteri, rectum, skin and mouth has been previously described.[1–4] With the aid of appropriate instruments, this method has also been utilized in making available for biopsy, tissue from the mucosal surface of the rectosigmoid colon, bronchi and esophagus. In these locations, under direct visualization, the sponge has been rubbed on the surface to be examined. While a similar procedure might be carried out in the stomach with the aid of a gastroscope, the author has felt the need for a simpler method of obtaining tissue from the stomach which might be applied in the hospital or in the office.

Two methods have been used to introduce sponges into the fasting stomach. The test is begun shortly after 9:00 A.M., the patient having abstained from solid food and liquids for the previous 15 hours. In the first method, 9 to 12 small pieces of fine-pore specially-prepared synthetic cellulose sponge,† each 1.0 x 0.5 x 0.5 cm., are tied at the lower end of a piece of No. 3 white braided silk at intervals of 2.5 cm. (Fig. 1). The lower end is weighted with a small metal bulb, such as the Rehfuss duodenal bulb. In the second procedure, 12 to 15 small pieces of sponge are tied at similar intervals along braided silk and then firmly wound in spirals along the lower portion of a Levine stomach tube for a distance of 30 cm. (Fig. 1). A small hole in the tube, 30 cm. above the lower end, will permit passage of the silk through the lumen, and firm ligature of the two free ends will prevent slipping. The sponges, string and tube are immersed in cold water before they are swallowed. A marker, 75 cm. above the lower end, indicates the approximate distance from the incisor teeth to the pylorus. After the sponges have reached the stomach the patient is asked to lie on the right side and the upper portion of the abdomen is massaged by moderately firm pressure. The patient then lies on his back and massage is repeated, the purpose being to bring the sponges into intimate contact with the gastric mucosa and with any ulcerative surfaces which may be present. The patient then sits erect. Gastric juice is aspirated by syringe through the tube, after which the tube and sponges are withdrawn. Sponges, braided silk, and adherent mucoid or particulate matter are immediately placed in a small bottle of 10 per cent formalin. The time interval between the swallowing of the sponges and their immersion into formalin should be as short as possible and need not exceed five minutes. Of the two procedures, we prefer the use

* Received for publication, May 31, 1949.

† The sponge used in these experiments was prepared with the collaboration of the Dupont Laboratories. It has been designated Onkosponge No. 1, and is available through Histomed, Incorporated, 181 Lafayette Street, Paterson, New Jersey.

of the tube since it facilitates swallowing and permits aspiration of gastric juice. The sponges are embedded, cut and stained as described in previous publications.

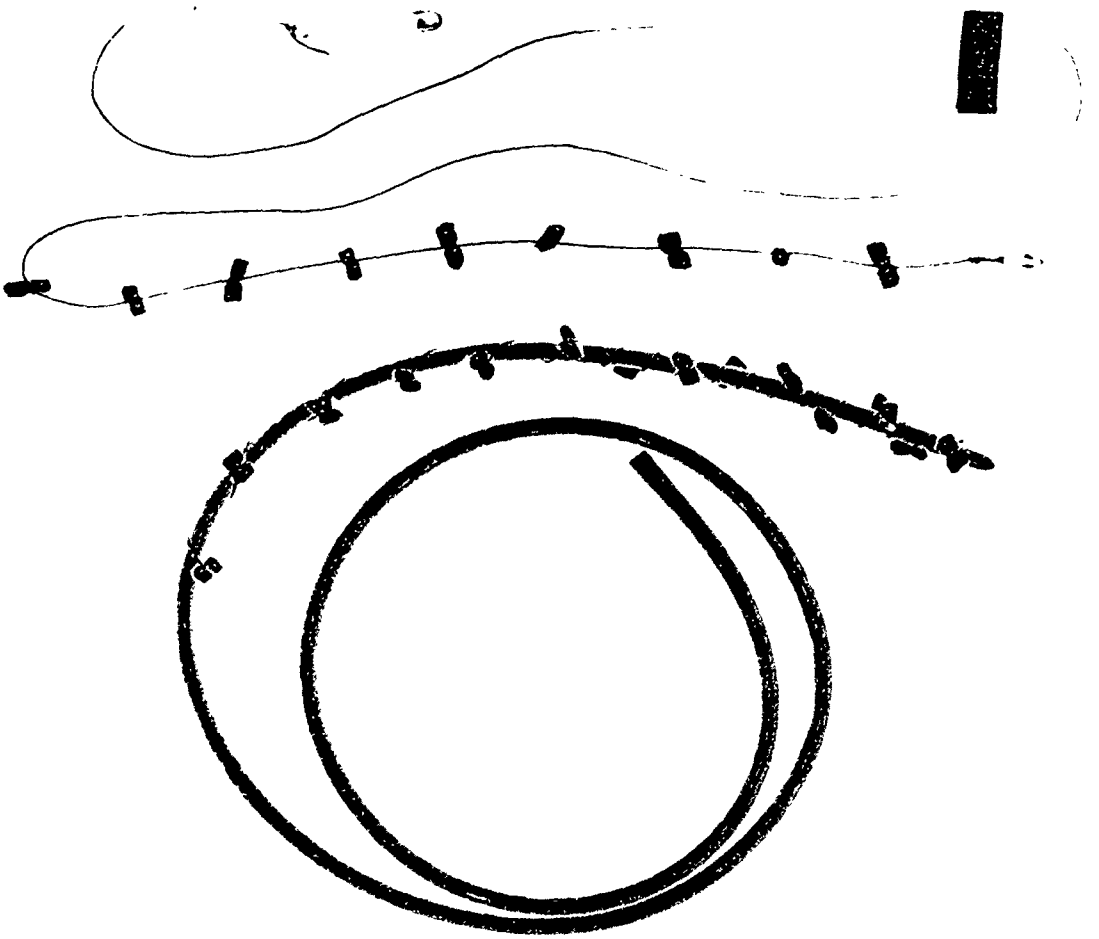


FIG. 1. Above, nine small pieces of sponge are tied at intervals along the lower end of a length of braided silk, weighted to facilitate swallowing. Below, 14 pieces of sponge are tied on braided silk and wound spirally around the lowest 30 cm. of a Levine stomach tube for introduction into the stomach.

The particulate matter separated from the gastric juice by filtration is similarly treated.

The type of material which may be gathered by sponge for biopsy in cases of cancer of the stomach is illustrated in the photomicrographs. Figure 2 is from a

FIG. 2. Case 4011, diagnosis, cancer of stomach. Particles of cancer tissue are composed of large darkly staining tumor cells showing much variation in size and shape. The smaller nuclei of inflammatory cells are seen in the periphery. × 400.

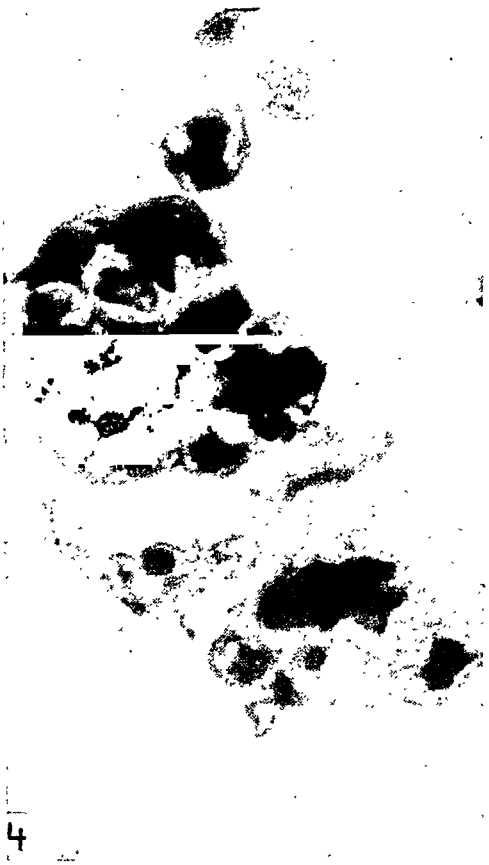
FIG. 3. Case 4008, diagnosis, cancer of stomach. A large particle of viable cancer tissue is seen. × 400.

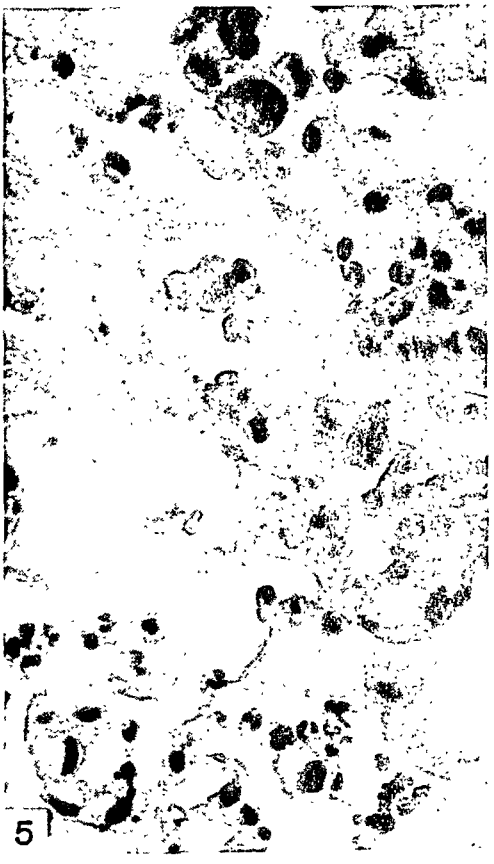
FIG. 4. Case 4008, diagnosis, cancer of stomach. Particles of cancer tissue are made up of large darkly staining atypical cells. × 400.

FIG. 5. Case 4014, diagnosis cancer of stomach. Groups and isolated cancer cells including signet ring cells are seen together with inflammatory cells. × 400.









FIGS. 2-5

case of gastric carcinoma proved by later exploratory laparotomy. The particles of cancer tissue are made up of large dark-staining atypical cells, varying markedly in size and shape. The nuclei of the tumor cells are two to four times as large as the nuclei of the inflammatory cells in the margins of the photograph. Figures 3 and 4 are from another case of gastric carcinoma. The large particle of tissue was one of several adherent to the sponge and was processed along with the sponge as previously described. (Particulate matter adherent to the sponges or free in the formalin often provide the best specimens and should be carefully looked for by the technician preparing the sections.) Here again the tumor cell nuclei show the characteristic large size and marked variability in size and shape.

The diagnosis of gastric carcinoma was established clinically and by x-ray examination in the first two cases before tissue was obtained from the stomach for biopsy. In the third case, however, a diagnosis of peptic ulcer had been made two years previously by means of x-ray films. Because of weakness and loss of weight, x-ray examination was repeated. This revealed rigidity of the lesser curvature of the stomach which suggested an infiltrative process, probably malignant. Material was then obtained for biopsy by the sponge method and showed abundant mucus, leukocytic exudate, and particles of tissue made up of large cells with large irregular nuclei, and abundant clear mucoid cytoplasm (Fig. 5). Characteristic signet-ring cells were also seen which supported a diagnosis of colloid carcinoma of the stomach. The stomach, removed surgically, showed an ulcerating and infiltrating colloid carcinoma of the lesser curvature.

This method is a comparatively simple one for obtaining, for microscopic examination, small particles of viable and well-preserved tissue from the surface of an ulcerating gastric lesion. At times it should be useful in establishing the diagnosis of cancer of the stomach. A more precise statement regarding its value in the diagnosis of cancer of the stomach or of other pathologic conditions of the gastric mucosa, must await the accumulation of a large body of experience.

REFERENCES

1. GLADSTONE, S. A.: Sponge biopsy in cancer diagnosis. Am. J. Med., **5:** 849–852, 1948.
2. GLADSTONE, S. A.: Use of sponge in the collection and preparation of tissue for biopsy. Am. J. Clin. Path., **19:** 92–94, 1949.
3. GLADSTONE, S. A.: Sponge biopsy—a new method in the diagnosis of cancer. Cancer, **2:** 604–614, 1949.
4. GLADSTONE, S. A.: Diagnosis of early carcinoma of the cervix by sponge biopsy. New England J. Med., **241:** 48–52, 1949.

NEW METHYLENE BLUE AS A RETICULOCYTE STAIN*

GEORGE BRECHER, M.D.

From the National Institutes of Health, Bethesda 14, Maryland

Brilliant cresyl blue (Color Index 877) is at present the only dye extensively used for the staining of reticulocytes. Different lots of this dye vary considerably in their staining properties. On spectrophotometric analysis, the absorption maxima of 10 lots varied from 615 to 636 mμ. There was no correlation between the staining performance of a given sample and the position of its absorption maximum. During an investigation of basic dyes, we found new methylene blue useful in the staining of reticulocytes. In contrast to brilliant cresyl blue, various lots of new methylene blue showed marked uniformity. The absorption maxima of 5 lots from 5 manufacturers varied from 630 to 632.5 mμ, and the samples gave identical results in staining tests. It should be noted that new methylene blue (Color Index 927) is chemically different from methylene blue (Color Index 922),[1] which is a poor reticulocyte stain.

METHOD

The staining solution consists of

New methylene blue	0.5 Gm.
Potassium oxalate	1.6 Gm.
Distilled water	100.0 ml.

Approximately equal amounts of staining solution and fresh or oxalated blood are mixed on a slide. The mixture is drawn up into a capillary pipet, allowed to stand for 10 minutes, expelled on a slide, and mixed again. Thin smears are then made and air-dried. Reticulocytes are counted under oil immersion without fixation or counterstaining. The red blood cells are light greenish blue in these preparations. The reticulum is sharply outlined, and deep blue.

The staining of the reticulum is not affected by relatively wide variations of the proportion of blood and staining solution. However, the proportion of blood and staining solution determines the color of the red cells. A marked excess of staining solution produces a deep blue color of the red blood cells, obscuring the reticulum. A large excess of blood diminishes the greenish-blue coloration of the red blood cells. Generally a slight excess of blood gives optimal color contrast.

Occasionally the mixture of blood and staining solution will dry out at the tip of the pipet and occlude it. This occurs if a very fine capillary pipet has been used or if the mixture has been allowed to stand for a prolonged period of time. In that case the tip of the pipet may be broken off and the remainder of the procedure followed as outlined above, without affecting the reticulocyte count.

Preparations may fade on storage. The original appearance of the reticulum can be restored by fixation in methyl alcohol and restaining with Wright's or Giemsa's stain.

* Received for publication, July 1, 1949.

Parallel counts on human, dog, rabbit, rat and mouse blood, using the new methylene blue method and the brilliant cresyl blue technics recommended by Dameshek,[2] Sanford,[4] and Wintrobe,[5] showed excellent agreement.

DISCUSSION

The staining solution used has the advantage of simplicity. Potassium oxalate serves both to prevent coagulation and to render the staining solution isotonic. In addition, dry smears prepared with this stain can be counted easily without counterstaining, because all red cells are made clearly visible by a light greenish-blue color.

Dry smears are preferred to wet preparations because they may be counted when convenient and slides may be kept on file. Moreover, Nizet and Govaerts[3] have shown that reticulocytes are distributed uniformly in dry smears and that counts made upon random fields vary only within the limits of the statistically expected error. On the contrary, the observations of Dameshek[2] indicate that wet smears must be counted in suitably selected fields to give consistent results.

SUMMARY

New methylene blue (Color Index 927) was found superior to brilliant cresyl blue as a reticulocyte stain because of its uniform performance and the sharp, deep blue staining of the reticulum, which facilitates identification and enumeration of reticulocytes.

Acknowledgment. My thanks are due to Mrs. Helen A. Grimes for skillful technical assistance.

REFERENCES

1. Conn, H. T., Biologic Stains. Ed. 5. Geneva, N. Y.: Biotech. Publications, 1946.
2. Dameshek, W.: A method for the simultaneous enumeration of blood platelets and reticulocytes. Arch. Int. Med., **50**: 579–589, 1932.
3. Nizet, A., and Govaerts, T.: Etude critique des techniques de numération des réticulocytes. Application du calcul des erreurs. Sang, **18**: 466–474, 1947.
4. Todd, T. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods. Ed. 10. Philadelphia: W. B. Saunders Co., 911 pp., 1943.
5. Wintrobe, M. M.: Clinical Hematology. Ed. 2. Philadelphia: Lea and Febiger, 862 pp., 1946.

AN INSTRUMENT TO LEVEL TISSUE IN PARAFFIN EMBEDDING*

MILDRED MILLIGAN, A.B.

From the Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, Illinois

In preparing paraffin sections, some difficulties encountered include: (1) Getting large blocks of tissue level enough to cut an entire section without loss of too much tissue in the trimming process, (2) orienting of friable tissue, and (3) obtaining representative sections of all pieces of tissue, as in curettings. To obviate these difficulties the instrument illustrated in Figure 1 was devised.

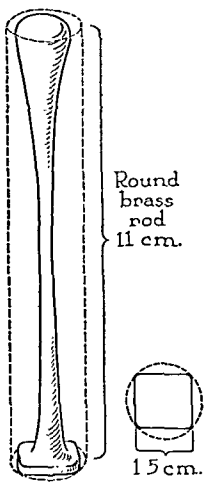


FIG. 1

The instrument was turned on a lathe from a bar of brass 11 cm. in length and 2.0 cm. in diameter. The base was filed, 1.5 cm. square, then smoothed and polished. This square base fits more efficiently into corners of the embedding pans or molds, either metal L's, paper boxes or plaster boats. Tissue is placed in the mold as usual; instead of pressing down with the end of a heated forceps, the plunger is heated, gently forced against the tissue, and moved along the entire block. In this way, the tissue is flattened regardless of the length of the block. The instrument is especially useful when working with friable tissue, such as placenta or brain tissue. In applying pressure with this rod, one does not break or push through the block as he is likely to do with forceps.

Large blocks of tissue, which frequently are quite useful both for diagnosis and teaching may become distorted during the dehydration and infiltration. However, with this brass plunger it is possible to flatten such tissues without injury.

The instrument has been used in our laboratory for several years with satisfying results.

* Received for publication, March 4, 1949.

TRICHROME STAIN FOR TISSUE SECTION, CULTURE OR SMEAR*

MACHTELD E. SANO, M.D.

From the Department of Tissue Culture and Research Pathology, Temple University Medical School and Hospital, Philadelphia, Pennsylvania

It has been found that Harris' hematoxylin followed by Pollak's trichrome mixture can be used successfully not only for paraffin sections but for tissue cultures and cytologic smears as well. For best results, tissue cultures must be fixed in *ether-alcohol* instead of formalin-trichrome solution.

The usual method of fixation is followed for tissue sections and smears. Tissue sections are not differentiated after hematoxylin in acid; tissue cultures and smears are.

STAINING OF TISSUE CULTURE AND SMEARS

1. Dilute hematoxylin (0.25 per cent), six minutes
2. Three dips in distilled water
3. Six dips in 0.5 per cent hydrochloric acid
4. Running tap water (filtered), six minutes
5. Trichrome mixture, six minutes
6. Two dips in 0.2 per cent acetic acid in distilled water
7. Dehydrate through 95 per cent and absolute alcohol followed by two changes of xylol

STAINING OF PARAFFIN SECTIONS

Deparaffinize, then carry through the alcohols up to the distilled water. Then:

1. Hematoxylin (0.5 per cent), one-half minute, according to batch of stain
2. Running tap water, 20 minutes, then steps 5, 6, 7, as above. The paraffin sections can be run through the 0.5 per cent hydrochloric acid and the washing shortened, but this does not give uniformly good results in our experience.

Acknowledgments. I am indebted to Dr. E. S. Gault for his criticism of the stained tissue cultures and paraffin sections, and to Carol Bocher, B.S., and Willy Koetsier, B.S., for their assistance in developing this technic.

REFERENCES

1. POLLAK, O. J.: A rapid trichrome stain. Arch. Path., **37**: 294, 1944.
2. SANO, M., AND SMITH, L. W.: A simplified method for quantitative tissue culture in vitro. Proc. Soc. Exper. Biol. and Med., **28**: 282–284, 1930.

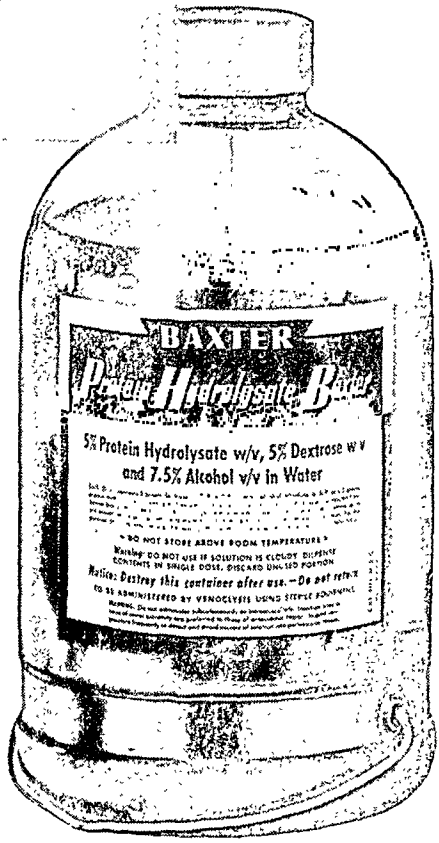
* Received for publication, February 18, 1949.



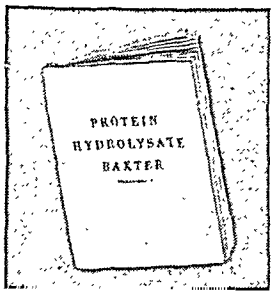
Announcing

an aid to

Sedation



Many investigators have reported the value of parenteral alcohol in operative cases as a sedative and analgesic, substituting for opiates and so eliminating undesirable side effects. In introducing this solution of 5% Protein Hydrolysate w/v, 5% Dextrose w/v, and 7.5% Alcohol v/v, Baxter Laboratories offer a single solution which provides protein nourishment, caloric value and sedation.



Write today for 16-page booklet giving detailed information on Protein Hydrolysate, Baxter.

product of
BAXTER Laboratories
Morton Grove, Illinois
Acton, Ontario

protein and caloric requirements with minimum fluid infusion In many cases where nutrition must be provided parenterally, it has been difficult to provide sufficient calories without too much fluid. In addition to its analgesic value, this solution provides a richer available source of energy than dextrose and protein digest solutions. Each 1000 cc. provides 800 calories.

Distributed and available only in the 37 states east of the Rockies (except El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION

GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

A POSTGRADUATE COURSE IN

CLINICAL PATHOLOGY

FOR

LABORATORY TECHNICIANS

December 1-3, 1949

Offered By

WASHINGTON UNIVERSITY

SCHOOL OF MEDICINE

Tuition $25.00

The course is designed as a period of postgraduate training for laboratory technicians. Recent advances in clinical pathology will be reviewed.

For further information write:

Director, Division of Postgraduate Studies

Washington University School of Medicine
4580 Scott Avenue
St. Louis 10, Missouri

FOR BEST RESULTS
IN URINALYSIS USE
USSLA
Products

Krautman's:

ALBU-MI-TEST TABLETS — A direct, Simple, and Rapid Method for the Detection of Albumin in Urine
250 Tablets $1.95

SULFA-TEST TABLETS—A Rapid and Accurate Method for the Detection of Sulfonamide Drug or Crystals in Urine.
100 Tablets $1.50

ACETONE-TEST POWDER—For the Accurate, Simple, and Rapid Detection of Acetone in Urine.
12 vials 10 gms. each $7.25

No Liquid Reagents, Heating or Pipetting

Literature for USSLA Products on request.

U. S. Scientific Laboratories
237 Christopher Ave. Brooklyn 12, N. Y.

Special Announcement

The Board of Registry will meet in Chicago on October 8 and 9, 1949. If you have any special problems to present for the Board's consideration, please direct your letters to the Registry office, or to individual members of the Board, whose names and addresses are listed below. It is only by close cooperation with members of the Society that the Board is able to discharge its responsibilities.

LALL G. MONTGOMERY, M.D.
Chairman
Ball Memorial Hospital
Muncie, Indiana

A. G. FOORD, M.D.
Huntington Memorial Hospital
Pasadena, California

FRANK B. LYNCH, JR., M.D.
Germantown Dispensary and Hospital
Philadelphia, Pennsylvania

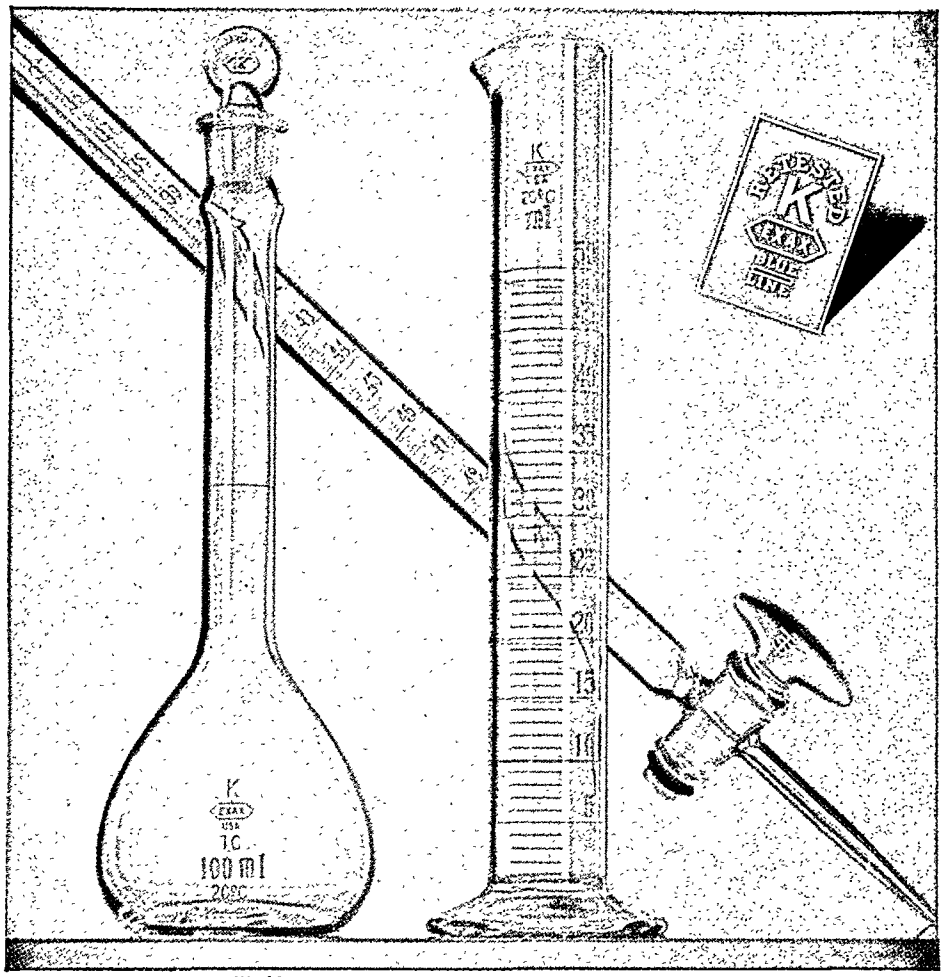
FRANK B. QUEEN, M.D.
University of Oregon
Medical School
Portland, Oregon

RALPH G. STILLMAN, M.D.
315 East 68th Street
New York, New York

A. H. WELLS, M.D.
St. Luke's Hospital
Duluth, Minnesota

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
American Society of Clinical Pathologists
Muncie, Indiana

(In writing to advertisers, please mention the Journal—it helps.)



Kimble EXAX RETESTED Flask No. 28015; Cylinder No. 20030; Burette No. 17030.

There's no substitute for EXAX RETESTED quality

EXAX RETESTED is America's No. 1 choice for accurate laboratory determinations simply because it is made and intended for that purpose. EXAX won't bounce, but it is thoroughly annealed for toughness . . . will stand everyday handling and usage.

The great value of EXAX RETESTED lies in certain exclusive advantages. It is a special formula of glass, precision-made by specialty craftsmen.

The fine deep graduations are of a type which readily accepts the permanent fused-in blue filler. *And* . . . all calibrations are marked and checked and *retested* with standards of highest precision.

So remember, if accuracy and fitness for the job are important, there is no substitute for EXAX RETESTED glassware.

Look for the Kimble "K", the visible guarantee of invisible quality.

KIMBLE GLASS TOLEDO 1, OHIO

Division of Owens-Illinois Glass Company

LABORATORY TECHNIQUE

In Biology and Medicine

SECOND EDITION

By E. V. Cowdry

Professor of Anatomy, Washington University

An excellent laboratory instrument for the technician

- Expanded to include related physical methods and micro-chemical technique.
- New methods and improvements in standard techniques added.
- A laboratory tool that broadens the utility of every other piece of laboratory equipment in its scope.
- It is "definite information on specific matters," as the author states.
- And the definite information is really accessible—all alphabetically arranged under names of structures, and strains, and elements, and names of procedures. You can hardly miss.
- Thoroughly documented with the references where you want them—as part of the text you are reading at the moment.
- In short, as the *American Journal of Medical Technology* puts it, the worker "will find it useful at the work table instead of on the book shelf."

LABORATORY TECHNIQUE

Cloth 6 x 9 **224 pages** **self-indexing** **$4.00**

THE WILLIAMS & WILKINS COMPANY

Mt. Royal & Guilford Aves.

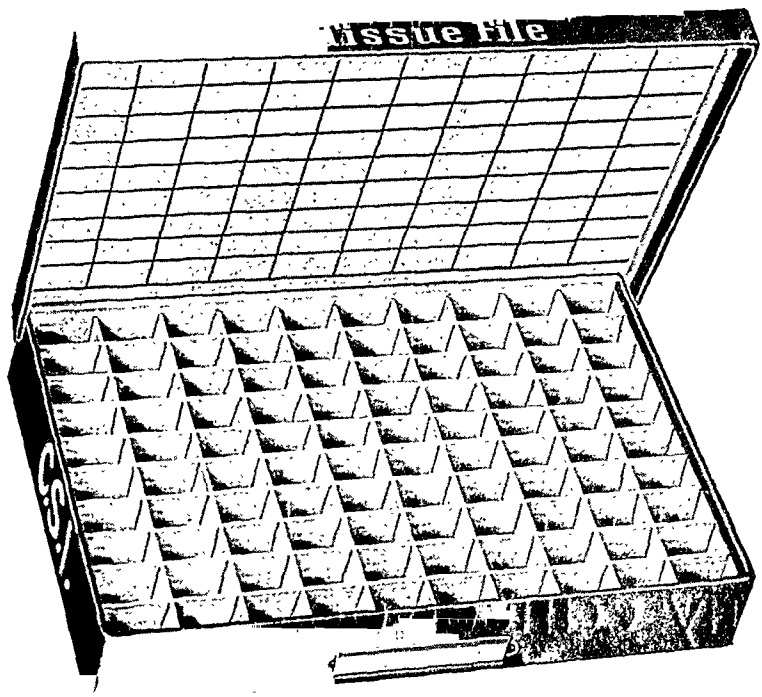
Baltimore 2, Maryland

The Cargille

TISSUE FILE

For filing and storing Histology Tissues Embedded in Paraffin or Celloidin Blocks.

Will also prove indispensable in anatomy and other biological laboratories where tissues are studied by histological methods.



- Conveniently stores one hundred groups of blocks in one case. Each compartment will accommodate 10 to 15 blocks of the usual size.
- Removable partitions make it possible to double or quadruple the size of compartments.
- Indexing data (date, number, etc.) can be recorded on card in front of case.
- Inside of hinged cover is ruled into spaces corresponding to compartments. Data for indexing each lot of specimens can be recorded in proper space for instant reference.
- Sturdily constructed, specially reinforced. Designed so that each case can be conveniently numbered and stacked on shelves.

● Case measures $15\frac{1}{4}'' \times 10\frac{1}{2}'' \times 2\frac{3}{4}''$ and is divided into 100 compartments, each measuring $1\frac{1}{2}'' \times 1'' \times 2\frac{1}{4}''$ deep. Shipping weight for each case $3\frac{1}{2}$ lbs. PRICE, 1 to 11 . . $2.75 ea; 1 doz. $27.50

CARGILLE SCIENTIFIC INC.

118 LIBERTY STREET NEW YORK 6, N. Y.



WHITE MICE
WHITE RATS



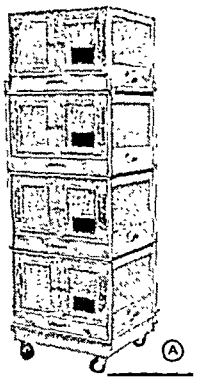
RESEARCH SUPPLY CO.
2436 W. York Street
Philadelphia 32, Pa.

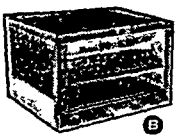
BUILT ESPECIALLY
FOR LABORATORY USE . . .

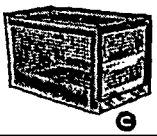
Wes-Bilt CAGES
AND RACKS

Light-weight, yet ruggedly constructed. Stainless steel pans and floor bindings are standard equipment. Special units designed and made to order. Write today for complete descriptive folder, or state your requirements.

A. Animal cages and rack.
B. Small animal cage.
C. Small animal cage.
D. Slant front cage.
E. Cage rack.



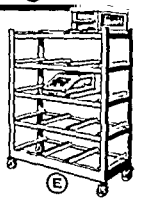








Wes-Bilt MANUFACTURING CO. INC.
[illegible] SOTO STS. HAYWARD, CALIF.



the papanicolaou stains for early cancer detection

Early cancer diagnosis by cytologic methods is now widely recognized as a most useful procedure. Accurate diagnosis of pelvic malignancy in women is possible (in the hands of competent experts) with the Papanicolaou Method of obtaining, fixing, and staining utero-vaginal smears. A total diagnostic error of only 2.8% has been reported in a series of 3,710 cases.

Successful application of the Papanicolaou cytologic method of cancer diagnosis to other body fluids including pleural, peritoneal, prostatic, bronchial, and gastric secretions has been widely reported and is discussed in detail in several articles appearing in the April 1949 issue of the American Journal of Clinical Pathology.

Literature Sent on Request

available:

Papanicolaou Stains EA 50 and OG 6 in 100 cc. and 450 cc. bottles.

EA 65 in 100 cc. bottles.

Harris Hematoxylin (Ortho) 100 cc. and 473 cc. bottles.

Vaginal Pipettes and Aspirator Bulbs for obtaining vaginal smears.

references:

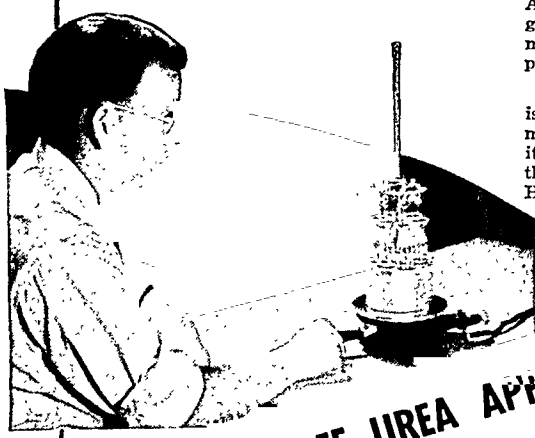
Papanicolaou, G. N., Am. J. Clin. Path. 19:320-322, 1949

Fremont-Smith, N. et al. New Eng. J. Med. 238:179-181, Feb., 1948

Ortho Pharmaceutical Corporation

Raritan, New Jersey

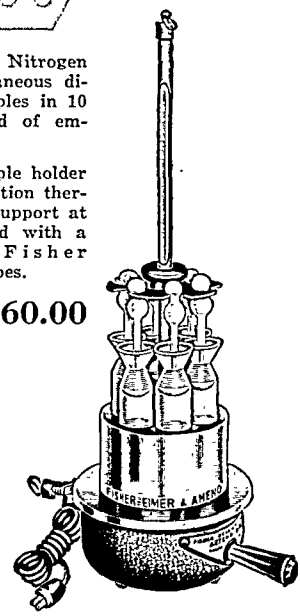
FISHER - E. & A. Apparatus for Urinalyses . . .



The E.&A.-Leiboff Urea Nitrogen Apparatus permits simultaneous digestion of one to six samples in 10 minutes—without the need of employing an autoclave.

The cast aluminum sample holder is provided with a combination thermometer shield and tube support at its center; it is furnished with a thermostatically-controlled Fisher Heater. With 2 Leiboff tubes.

$60.00



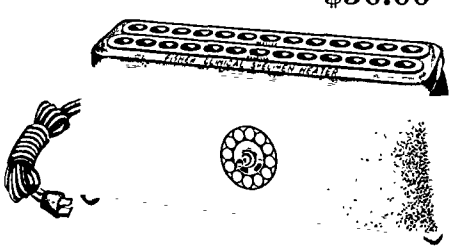
E. & A. LEIBOFF UREA APPARATUS

Thirteen urine samples for the Purdy and the Benedict tests—a total of 26—can be heated simultaneously in the Fisher Clinical Specimen Heater.

Standard size test tubes in the front row are heated only at the topmost inch for albumen testing; those in the rear row are base-heated for sugar determinations.

Fisher Clinical Specimen Heater, with thermostatically-controlled heating elements for 110 volt, A.C.

$30.00



FISHER CLINICAL SPECIMEN HEATER



Available with other Clinical Specialties from:

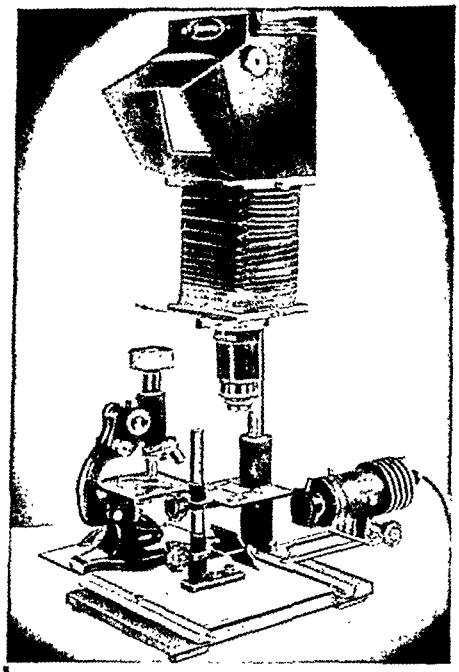
Headquarters for Laboratory Supplies

FISHER SCIENTIFIC CO.
717 Forbes St., **Pittsburgh (19), Pa.**
2109 Locust St., **St. Louis (3), Mo.**

EIMER AND AMEND
Greenwich and Morton Streets
New York (14), New York

In Canada: Fisher Scientific Co., Ltd., 904 St. James Street, Montreal, Quebec

(In writing to advertisers, please mention the Journal—it helps.)



Change From LOW POWER TO HIGH POWER IN 1 SECOND

With the New
Quick Change Base Plate

Another Exclusive with GAMMA Photomicrographic Cameras

For Descriptive Literature Write to

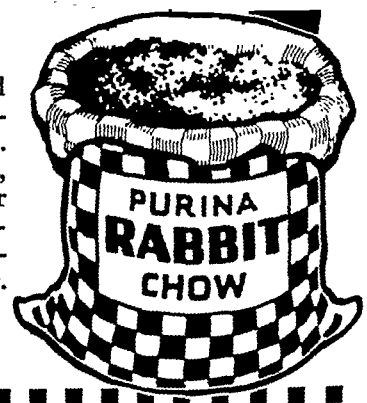
GAMMA INSTRUMENT CO., INC.

261-263 Great Neck Rd. Great Neck, L.I., N.Y.



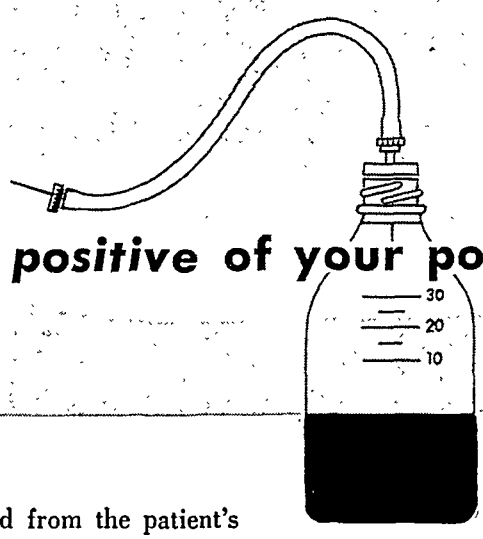
HERE'S WHY IT'S SO EASY TO FEED PURINA RABBIT CHOW

Blended into Purina Rabbit Chow are the food essentials for rabbit health and vigor . . . providing good condition for your experimental work. When you have an easy source of minerals, fat, protein, vitamins and carbohydrates, it's easy for you to feed your animals. No hay . . . no vegetables . . . nothing is needed when you feed complete Purina Rabbit Chow Checkers except water. See your Purina Dealer today.



RALSTON PURINA COMPANY
St. Louis 2, Mo.

NOW



you can be *positive* of your positives

Direct delivery of blood from the patient's vein, through a closed, sterilized system, into Trypticase Soy Broth media in the new B-D VACUTAINER CULTURE BOTTLE . . .

- **Provides *positive* positives and valid negatives**
- **Prevents outside contamination**
- **Avoids incorrect selection and multiplicity of media**

Aerobic or anaerobic conditions may be obtained at time blood specimen is taken. Bottle reaches laboratory ready for immediate incubation . . . speeds laboratory handling . . . provides faster diagnosis. Microaerophiles grow luxuriantly.

Write for descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE and learn how easy it is to add this new element of assurance to your blood culture procedures.

with the B-D VACUTAINER® CULTURE BOTTLE

containing Trypticase Soy Broth with CO_2 added

Prepared and sterilized by Baltimore Biological Laboratories, Inc., Baltimore, Md.

BECTON, DICKINSON & CO., Dept. 27-J
15 Ames Avenue,
Rutherford, N. J.

Please send me descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE that takes the guesswork out of blood culture procedures.

NAME_____

ADDRESS_____

CITY_____ ZONE____ STATE_____

BECTON, DICKINSON & CO., RUTHERFORD, N. J.

(In writing to advertisers, please mention the Journal—it helps.)

NEW

HELLIGE

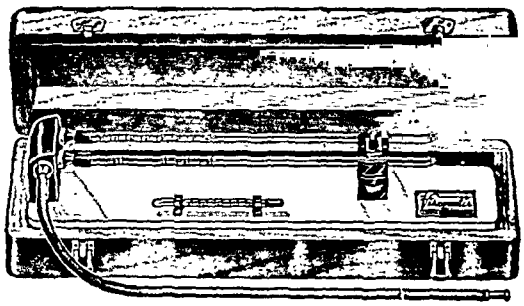
Viscometer

IMPROVED

HESS

BLOOD

VISCOSIMETER



The Hellige Viscometer is the first American-made instrument embodying improvements in the original Hess Blood Viscosimeter. Completely redesigned to provide greater simplicity and ease of operation, the Hellige Viscometer assures viscosity determinations of greater accuracy than were obtainable with the Hess model.

One of the new features is the viscometer tube, consisting of collecting, flow, and measuring portions sealed together in one complete unit, which can be handled as an ordinary pipette.

The accuracy of Hellige Viscometers is increased by addition of a special leveling device which eliminates the zero drift responsible for most of the erroneous determinations obtained with Hess Viscosimeters.

COMPLETE, $45.00

WRITE FOR CATALOG No. 1200

HELLIGE

INCORPORATED

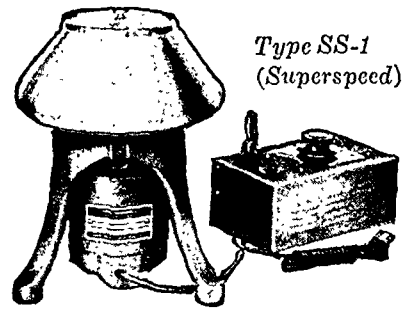
3718 NORTHERN BLVD.

LONG ISLAND CITY 1, N.Y.

SERVALL

ANGLE CENTRIFUGES

Ask for Bulletin J-91
with complete data on all models

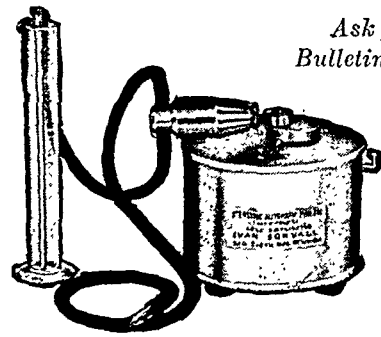


Type SS-1
(Superspeed)

With safety design self-centering device and dynamical balance. 13,000 r.p.m. 20,000 × G. Cap. 8 × 50cc or 15cc tubes.

NEW TYPE SS-2 VACUUM CENTRIFUGE
16,000 r.p.m. 45,000 × G.
Capacity 16 × 50cc or 15cc tubes

Ask for Bulletin J-92



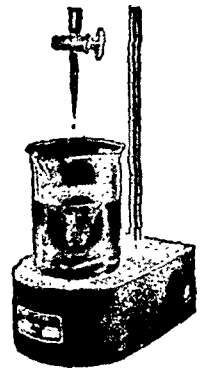
STERLING AUTOMATIC

AND

HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.

Easy to Operate—Time Saving



SERVALL

Magnetic Stirrer

Ask for Bulletin J-94

For stirring in open vessels or in closed systems under any pressure or under sterile conditions. Makes possible central rotation with vortex formation, epicyclic motion without vortex, simultaneous stirring in several small containers, etc.

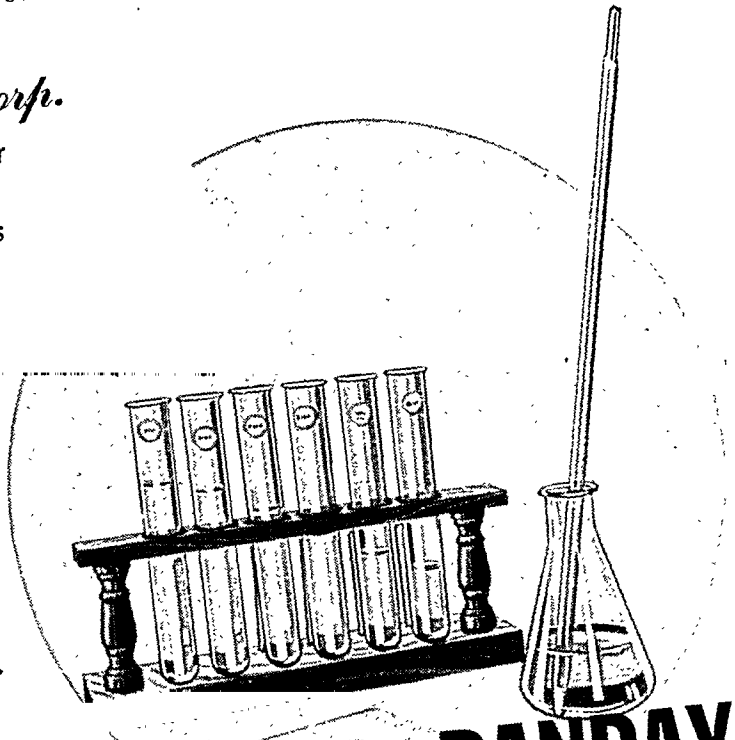
Manufacturer and Distributor:

IVAN SORVALL, Inc.

210 FIFTH AVE.-NEW YORK 10, N.Y.

The Panray Corp.

pioneer manufacturer
of DOPA and other
specialized chemicals
for investigation
and research...



announces the
availability of...

IODOACETIC ACID, PANRAY

TWICE RECRYSTALLIZED, SPECIAL REAGENT GRADE

for the Iodoacetate Index Determination of Serum Proteins

DOPA "PANRAY"
(3,4-dihydroxyphenylalanine, C. P.)
available in l and dl forms

Synthetic chemical reagents ... capable of revealing the presence of an oxidase in cells elaborating melanin. Valuable indicators of active melanogenesis.

Iodoacetic acid is a recently reported reagent for the determination of *deficient thermal coagulation of serum proteins* in cancer and other pathological conditions.
Originally reported by C. Huggins, M.D., G. M. Miller, M.D., and E. V. Jensen, Ph.D.: Thermal Coagulation of Serum Proteins—II. Deficient Coagulation in Cancer and the Iodoacetate Index—*Cancer Research, Vol. 9, No. 3, March 1949.*

Write for prices and other pertinent data

The Panray Corp.

398 BROADWAY
NEW YORK 13, N. Y.

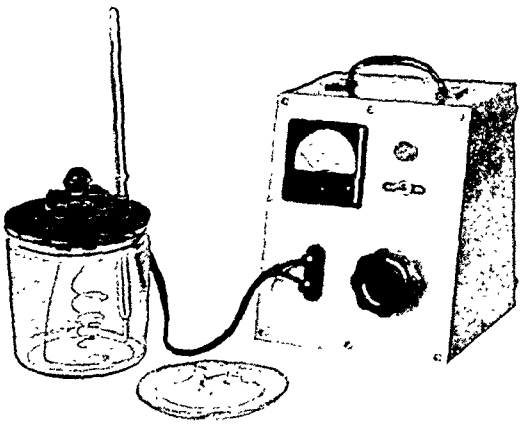
Custom Manufacturers of Fine Organic Chemicals

(In writing to advertisers, please mention the Journal—it helps.)

New

EBERBACH
BONE
DECALCIFIER

Prepares Bone Specimens
For Histologic Section



This new apparatus provides a rapid means of decalcifying bone for histologic section as described by I. M. Richman, M. Gelfand and J. M. Hill in "A Method of Decalcifying Bone For Histologic Section", July 1947, Vol. 44, pp. 92-95, *Archives of Pathology*. Apparatus includes a direct power source with an ammeter for indicating output and a knob for regulating this D. C. output between 0 and 6 amperes. The glass jar holding the electrolyte is fitted with a special cover for holding electrodes and thermometer. Write for Bulletin 180.

Eberbach & Son Company
LABORATORY APPARATUS & SUPPLIES
ESTABLISHED 1849
ANN ARBOR, MICH.

BBL

C. T. A. Medium
Cystine Trypticase
Agar

A basic medium for maintenance and determination of fermentation reactions of fastidious organisms, including members of the genus *Neisseria*. It does *not* require addition of ascitic fluid or serum.

Literature and prices sent on request.

BALTIMORE
BIOLOGICAL LABORATORY

1640 Gorsuch Avenue Baltimore-18, Md.

Labreco

Prepared Clinical Laboratory Reagents for

BIOCHEMICAL
BACTERIOLOGICAL
HISTOLOGICAL
SEROLOGICAL
HEMATOLOGICAL
PROCEDURES

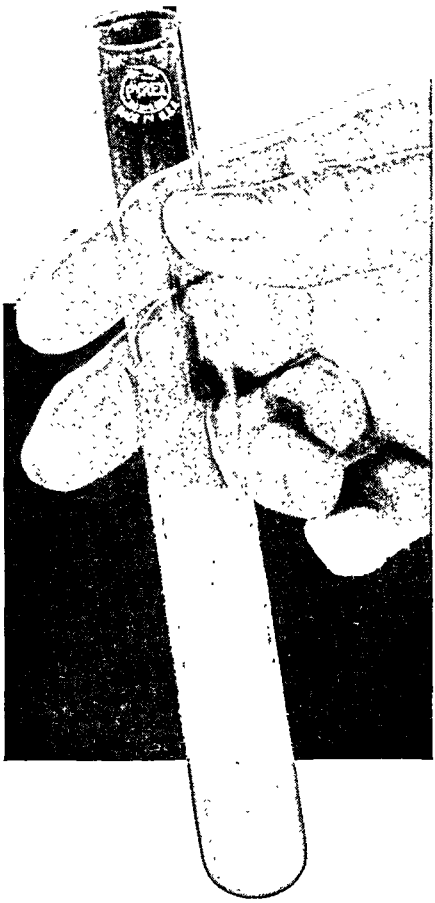
Prepared Bacteriological Culture Media in Sterile Screw Cap Test Tubes—
Petragnani T.B. Medium
One Dozen 2.75
Loeffler's Blood Serum
One Dozen 2.25

HEMATOLOGICAL AND BACTERIOLOGICAL
STAINS

CATALOG UPON REQUEST
LABORATORY REAGENTS
COMPANY
Woodbridge, New Jersey

(In writing to advertisers, please mention the Journal—it helps.)

3½ times the service with



T. M. REG. PYREX U.S. PAT. OFF.

TEST TUBES

Clinical research requires test tubes in large numbers. Consequently, their cost, service life considered, is a matter of importance. From this standpoint alone, you will find that PYREX brand test tubes are the best investment. Careful records maintained over a period of years by large test tube users show that PYREX tubes last 3½ times longer than other brands.

This is not so surprising when you consider that no other glass stands up like PYREX brand No. 7740. Compare it with any other laboratory glassware under identical conditions. The superiority of PYREX ware to chemical, thermal and physical resistance will be readily demonstrated.

All of this is reflected in more accurate analysis. Your laboratory dealer carries complete stocks of PYREX brand test tubes for your ordering convenience.

CORNING means research in GLASS

PYREX T.M. REG. U.S. PAT. OFF.

Stocked by Leading Laboratory Supply Houses

CORNING GLASS WORKS • CORNING, N. Y.

LABORATORY GLASSWARE

TECHNICAL PRODUCTS DIVISION · LABORATORY GLASSWARE • SIGNALWARE • GLASS PIPE • GAUGE GLASSES • LIGHTINGWARE • OPTICAL GLASS • GLASS COMPONENTS

(In writing to advertisers, please mention the Journal—it helps.)

WHY YOU SHOULD SPECIFY

"VACSEAL"

GUINEA PIG COMPLEMENT

Because it is a superior complement—the result of a decade of experience in guinea pig complement production, the proper choice and preparation of animals and the use of improved technics.

Because our large output enables us to pool the serum of no less than 100 guinea pigs per lot, assuring a more uniform titer. It also makes it possible for you to obtain a complete lot of any complement which has proved, through pretesting, to be particularly desirable for your purpose.

Because of the high vacuum and all glass containers, storage for at least two years is possible. To you this means economy and convenience.

"Vacseal" Complement is distributed in three sizes with a special diluent for restoration to the following amounts:

3 ml. 7 ml. 20 ml.

Any recognized laboratory will receive a trial 3 ml. sample upon request.

CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK

Advertisers in the Journal Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.



XENOPUS LAEVIS FROG

Pregnancy Diagnosis

Animal Costs Greatly Reduced

We assure you of a continuous supply. Accurate, speedy and inexpensive. Average animal cost per test, 20 cents to 35 cents. We carry a complete stock of Laboratory Tanks, Isolation Jars, Holders, and correct reagents and apparatus for running tests. Speedy delivery via AIR EXPRESS. Current brochure showing new low prices sent upon request.

Jay E. Cook, Importer

P. O. Box 302 Baltimore 3, Md., U.S.A.
Cable Address—XENOPUS-BALTIMORE

Reagents for Determining Prothrombin Time

Thromboplastin Extract Activity Chart Available upon Request

THROMBOPLASTIN
(Dehydrated Rabbit Brain)
6 ampuls (0.15 gram each)..... $4.50

THROMBOPLASTIN EXTRACT
(Desiccated)
1 c.c. ampul..... $1.00
6 ampuls (0.5 c.c. each)..... $4.00
10 individual test ampuls..... $2.00

SODIUM OXALATE SOLUTION
(0.1 Molar)
4 oz..... $0.75

CALCIUM CHLORIDE
SOLUTION (0.02 Molar)
4 oz..... $0.75

NORMAL PLASMA FOR
CONTROL (Desiccated)
1 c.c..... $1.00
6 ampuls (0.5 c.c. each)..... $4.00

CAPPEL LABORATORIES
Diagnostic Reagents
Box 352 Wayne, Pa.

(In writing to advertisers, please mention the Journal—it helps.)

for

BLOOD TYPING
RH TESTING

you need the highest-titred serum available.

ANTI-HUMAN PRECIPITIN SERUM (rabbit)
for the Coombs Test

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human Red Cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic Serum
for control tests

P.S.A.
(Paraffin Section Adhesive)

Write for complete information.

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York

(In writing to advertisers, please mention the Journal—it helps.)

LABORATORY C&B REAGENTS

Inorganic and
Organic Chemicals

•

Biological Stains
Solutions

•

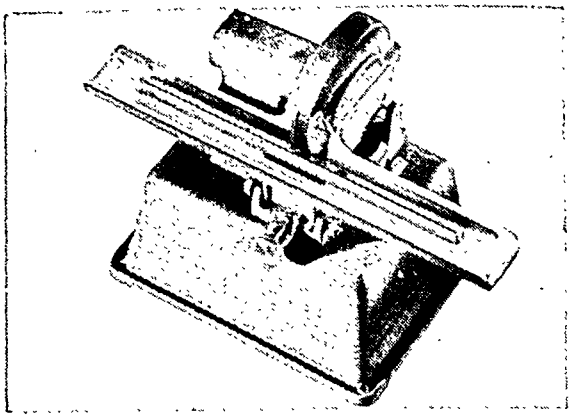
Chemical Indicators
Test papers

•

Write for copy of our new catalog
The COLEMAN & Bell Co., Inc.,
Manufacturing Chemists: Norwood, O., U. S. A.

COLEMAN & BELL

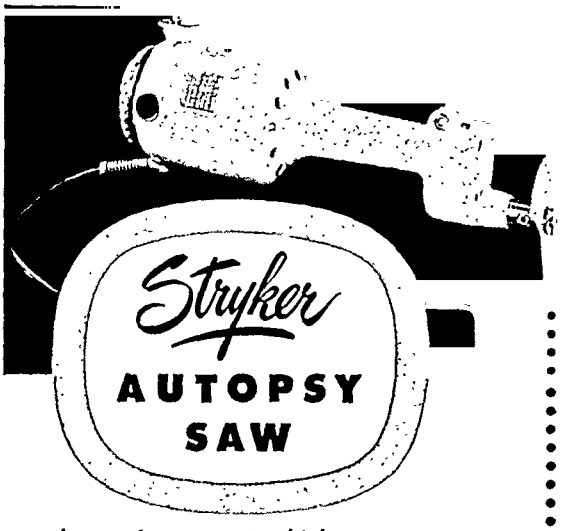
INDISPENSABLE
in your laboratory...



the Berkeley
MICROTOME KNIFE SHARPENER
for perfect edges...three times faster

Operation is simple, eliminates honing and stropping. Assures hollow-ground edges that cut better and last much longer. *Send for our Illustrated Bulletin MKS-14*

Berkeley Scientific Company
SIXTH AND NEVIN AVENUE • RICHMOND, CALIF.



A new instrument which simplifies bone cutting

Electrically driven, oscillates at high speed to cut bone efficiently with complete safety. Cutting blades do not hurl material. Two-sided blade can be adjusted to three positions. Blade, arbor and shaft are stainless steel. Write Department C for complete information.

ORTHOPEDIC FRAME COMPANY Kalamazoo Michigan

THOM *and* RAPER
A MANUAL *of the*
ASPERGILLI

Thorough Working Knowledge

The manual provides the mycologist and microbiologist with a means for identifying and interpreting any Aspergillus as it is isolated from nature, thus opening to him the entire literature. Every form that is found in the literature is enumerated and its proper allocation is indicated.

A summary outline of the exact observations to be made in describing an Aspergillus sets forth the complexities in the specific combinations of these characters found in the examination of moldy material and in the isolated colonies of individual strains. A complete index quickly leads to the information you are looking for.

573 pp. 315 illus. in 76 figs. $7.00

The Williams & Wilkins Co.
Mt. Royal & Guilford Aves • Baltimore 2, Md.

we say they're better

and so will you when you've

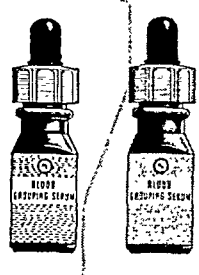
proved it in your own laboratory

HYLAND BLOOD GROUPING SERUM

Anti-A and Anti-B

Prepared from carefully selected donors to assure a highly potent, rapid acting, and specific product. Accurate results with maximum speed.

Available in 2 cc. and 5 cc. sets.



HYLAND ANTI-Rh SERUMS

Anti-Rh_0 (Anti-D) Typing Serum

Anti-Rh_0 rh' rh" (Anti-CDE) Typing Serum

Outstanding serum for slide method—rigidly tested for titer, avidity, specificity and sterility. Prepared from selected, immunized donors.

Both types available in 2 cc. and 5 cc. sizes.



make this test at our expense!

Clip the coupon for your free samples today! You'll receive generous samples of Rh_0 Typing Serum and Blood Grouping Serum. Enough to convince you that their speed, accuracy and titer are unsurpassed.

Hyland Laboratories

4534 Sunset Boulevard, Los Angeles 27, California

Please send me generous samples of Rh_0 and Blood Grouping Serums.

Your Name_____

Hospital or Lab. Name_____

Address_____

City and State_____

From what dealer do you regularly buy your typing serums?_____

(In writing to advertisers, please mention the Journal—it helps.)

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia.....O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Benbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

American Society of Clinical Pathologists

OFFICERS

President: Dr. O. A. Brines, Wayne University College of Medicine, Detroit 26, Michigan
Vice-President: Dr. C. H. Manlove, 5725 S.E. Stark Street, Portland 15, Oregon
President-Elect: Dr. J. B. McNaught, University of Colorado School of Medicine, Denver, Colorado
Secretary-Treasurer: Dr. C. G. Culbertson, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana

STANDING COMMITTEES

EXECUTIVE COMMITTEE

W. S. Thomas, Chairman, Rochester, N. Y.
T. J. Curphey, Long Island, N. Y.
F. W. Konzelmann, Atlantic City, N. J.
L. W. Larson, Bismarck, N. D.
S. P. Reimann, Philadelphia, Pa.
J. R. Schenken, Omaha, Neb.

BOARD OF CENSORS

L. W. Diggs, Chairman, Memphis, Tenn.
J. J. Andujar, Fort Worth, Tex.
F. C. Coleman, Des Moines, Iowa
J. W. Howard, Wilmington, Del.
V. M. Johnson, West Palm Beach, Fla.
C. P. Larson, Tacoma, Wash.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. Montgomery, Chairman, Muncie, Ind.
A. G. Foord, Pasadena, Cal.
F. B. Lynch, Jr., Philadelphia, Pa.
F. B. Queen, Portland, Ore.
R. G. Stillman, New York, N. Y.
A. H. Wells, Duluth, Minn.

APPOINTED COMMITTEES 1948-1949

GENERAL RESEARCH COMMITTEE

M. Strumia, Chairman
B. S. Kline
A. H. Sanford
W. M. Simpson

COMMITTEE ON HEMATOLOGIC TERMINOLOGY

E. E. Osgood, Chairman
L. Berman
R. R. Kracke
F. J. Heck
L. R. Limarzi

BLOOD BANK COMMITTEE

D. H. Kaump, Chairman
L. Y. Dyrenforth
L. W. Larson
F. W. Hartman
R. G. Stillman

COMMITTEE ON REGISTRY OF GENERAL TUMORS

J. W. Kernohan, Chairman
L. V. Ackerman
Col. J. E. Ash
W. A. D. Anderson
S. W. Lippincott
I. Perry

REPRESENTATIVE ON NATIONAL RESEARCH COUNCIL SUBCOMMITTEE ON ONCOLOGY

S. Warren

REPRESENTATIVE ON BOARD OF GOVERNORS OF COLLEGE OF AMERICAN PATHOLOGISTS

T. J. Curphey

COMMITTEE ON SEMINAR

I. Davidsohn, Chairman
P. R. Cannon
J. B. McNaught
Gen. R. O. Dart
R. A. Moore

PROGRAM COMMITTEE

C. G. Culbertson, Chairman
R. A. Moore, Co-Chairman
C. J. Bucher
F. B. Queen
F. W. Sunderman

COMMITTEE ON NECROLOGY

H. R. Brown

COMMITTEE ON EDUCATION AND PUBLICITY

W. O. Russell, Chairman
J. L. Carr
W. M. Germain
E. F. Hirsch
D. A. Nickerson
S. P. Reimann

COMMITTEE TO STANDARDIZE EDUCATION AND TRAINING OF MEDICAL TECHNOLOGISTS

J. J. Andujar, Chairman
F. C. Coleman
E. Moss
O. O. Christianson
M. Oosting
R. Miller
P. Rosenblatt
J. P. Tollman

DIFCO

Penicillin Inactivation

with

BACTO-PENASE

Bacto-Penase is a sterile thermolabile biological extract possessing the capacity to neutralize the antibiotic properties of all known types of *penicillin*. It is non-toxic to microörganisms and is therefore particularly satisfactory for use in the sterility testing of *penicillin* or products containing *penicillin*, and in determining the microbial counts on *penicillin*-containing products. BACTO-PENASE is recommended as a penicillinase for use in official procedures for sterility testing of *penicillin*. It is also recommended for use in media for culturing organisms from blood and other body fluids containing *penicillin* and for estimation of *penicillin* levels in such fluids.

PENICILLIN STERILITY TESTS

BACTO-PENASE in a concentration of 1 ml. per 15 ml. of Bacto-Fluid Thioglycollate Medium inactivates 50,000 units of *penicillin* in 40-48 hours incubation or 10,000 units of *penicillin* in 18 hours incubation.

BLOOD CULTURE

BACTO-PENASE is recommended for use in media for culturing blood or other body fluids containing *penicillin*. It is suggested that 1 ml. of BACTO-PENASE be added to 100 ml. of culture media such as Bacto-Brain Heart Infusion with P.A.B. or Bacto-Brain Heart Infusion with P.A.B. and Agar for this purpose. Media so prepared will inactivate the maximum amount of *penicillin* and sulfonamides present in 10 ml. or less of blood or other body fluids, and will also inactivate up to 1000 units of *streptomycin*.

℣ ℣ ℣

Stability. BACTO-PENASE and media containing this penicillinase are stable when stored at refrigerator temperatures (2°-6°C.).

Bacto-Penase Concentrate. BACTO-PENASE is available in concentrated form as BACTO-PENASE CONCENTRATE, 1 ml. of which neutralizes 500,000 units of *penicillin*. BACTO-PENASE CONCENTRATE is recommended in the sterility testing of *penicillin*.

BACTO-PENASE and BACTO-PENASE CONCENTRATE are supplied in standard packages of one-half dozen and one dozen screw cap tubes of 20 ml. each.

Descriptive literature is available upon request.

DIFCO LABORATORIES

DETROIT 1, MICHIGAN

VOLUME 19 APRIL 1949 NUMBER 4

AMERICAN JOURNAL OF CLINICAL PATHOLOGY

OFFICIAL PUBLICATION OF
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R. BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, 6060 Park Avenue, Indianapolis 5, Ind. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Agents: For Australia: Angus and Robertson, Limited, 89–95 Castlereagh Street, Sydney; for the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

VOLUME 19 AUGUST 1949 NUMBER

AMERICAN JOURNAL OF CLINICAL PATHOLOGY

OFFICIAL PUBLICATION OF
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R. BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pat

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devote publication of original investigations and observations in clinica pathologic anatomy. The JOURNAL is published monthly, one vol numbers appearing each year. The Journal includes a Technical Sect bi-monthly beginning with the January issue of each volume, which separately as the TECHNICAL BULLETIN. Manuscripts, books for review, ar inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten cop with *all* written matter, including references, double or triple spaced. Articl which are timely, and those which are presented concisely, will be given preferenc Manuscripts will be accepted for publication on condition that they are contribute exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References mu be listed in alphabetic sequence, each reference being arranged in the followin order: surname and initials of author, title of article in lower case except where capital letter is required, abbreviations of name of periodical (see *Index Medicus* volume in Arabic numerals, inclusive pages, year. References should be limited t articles mentioned in the text. The nomenclature for bacteria should be that give in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustr tions and tables, including legends and explanatory titles. Photographs preferabl should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper an should be unmounted. The magnification of photomicrographs should be state Illustrations must possess sufficient technical excellence to be acceptable. Th Journal will assume the cost of a limited number of illustrations in black and whit and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, sent with proof. No reprints are furnished gratis to contributors. Orders f reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICA PATHOLOGY is $8.00 per volume in the United States and countries within the post union; $8.50 in countries outside the postal union. Subscriptions of membe of the American Society of Clinical Pathologists are included in the membershi dues and are handled by Dr. C. G. Culbertson, Secretary, Indiana University Me ical Center, 1040–1232 West Michigan Street, Indianapolis 7, Indiana. The sul scription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cen additional per volume for countries outside the postal union. Subscriptions fro non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business m ters should be addressed to the publishers.

Publishers: The Williams and Wilkins Company, Mount Royal and Gu Avenues, Baltimore 2, Maryland, U. S. A.

Agents: For the British Empire, except Australia and Canada: Bai Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, Englan ustralia: Angus and Robertson, Limited, 89–95 Castlereagh Street, Sy fo Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

an Journal of Clinical Pathology

AUGUST 1949 NUMBER 8

CONTENTS

The Adhesiveness of Blood Platelets in Thromboembolism and Hemorrhagic Disorders. I. Measurement of Platelet Adhesiveness by the Glass-Wool Filter. S. E. MOOLTEN AND L. VROMAN 701

Quantitative Correlation of Morphologic Liver Changes and Clinical Tests. H. POPPER, F. STEIGMANN AND P. B. SZANTO 710

Sarcoidosis. A Clinicopathologic Review of Three Hundred Cases, Including Twenty-Two Autopsies. W. RICKER AND M. CLARK 725

Hashimoto's and Riedel's Strumas and Lymphadenoid Goiter. S. M. RABSON AND J. E. ARATA 750

Cytologic Diagnosis of Bronchogenic Carcinoma. L. B. WOOLNER AND J. R. MCDONALD 765

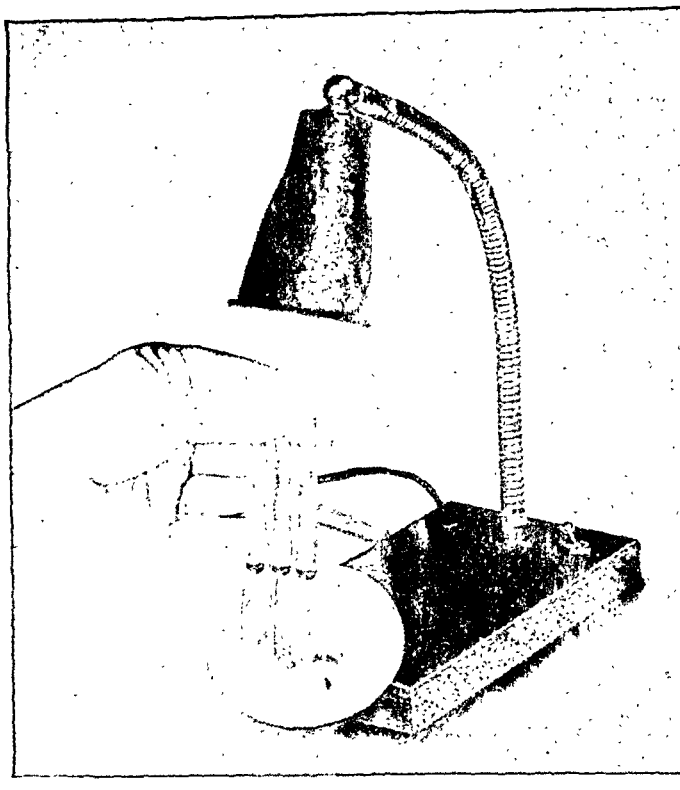
Progression from "Precancer" Stage to Early Carcinoma of Cervix within One Year. Combined Cytologic and Histologic Study with Report of a Case. J. E. AYRE AND W. B. AYRE 770

rther Studies on the rh^{w} Factor. A. S. WIENER, L. J. UNGER AND C. A. MAZZARINO 779

Abnormal Agglutinability of Red Cells in Pyogenic Infections. Report of Two Cases. G. ENGLESON AND R. GRUBB 782

linicopathologic Conference. J. D. KIRSHBAUM 789

ditorial: Pathways in Pathology. P. D. ROSAHN 796



for easier and more accurate reading

Test Tube Agglutination Viewer

The **Adams Test Tube Agglutination Viewer*** is designed for viewing test tubes in blood typing and cross-matching operations, for viewing Kahn tubes and for other sero-diagnostic, agglutination and flocculation test-tube procedures.

Test tubes and their contents are illuminated from above by a 25-watt shielded bulb and are viewed in an adjustable, magnifying mirror below. The mirror magnifies from 1½ to 2 times, depending on the distance of the test tube from the mirror.

The under surfaces of the test-tube contents are shown clearly in magnified form, permitting easier and more accurate reading than by use of a magnifying lens.

A-2330 **Adams Test Tube Agglutination Viewer,** with 25 watt bulb and magnifying mirror; finished in attractive gray hammertone **each $16.50**

*As suggested by Dr. Louis K. Diamond, Children's Hospital, Boston, for use in blood-typing tests.

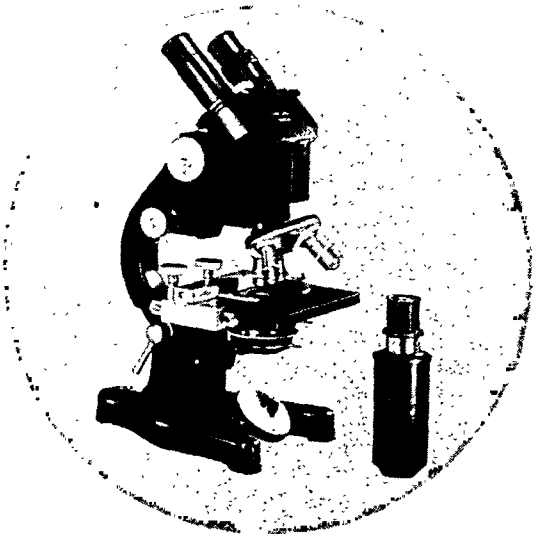
CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

(In writing to advertisers, please mention the Journal—it helps.)

Available now...

Leitz

Medical Microscopes



Leitz

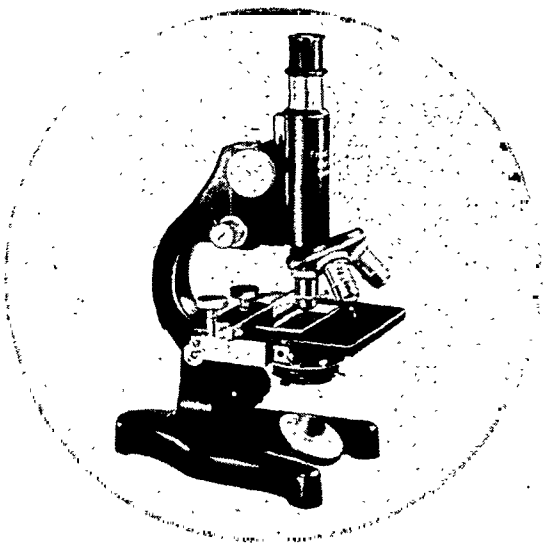
BINOCULAR MICROSCOPE BST 25/92

(Interchangeable monocular tube with draw tube available)

The ideal instrument for the medical profession offering:

Ball-bearing fine adjustment
Built-on, ungraduated mechanical stage
Quadruple nosepiece
3 Achromatic Objectives: 16 mm., 4 mm., 1/12th oil
2 pairs of eyepieces
Magnifications 60-1000X

$476



Leitz

MONOCULAR MICROSCOPE CT 25/92

The preferred student and laboratory microscope featuring:

Sturdy construction
Unsurpassed optical performance
Smooth-operating fine adjustment without gears
Built-on, ungraduated mechanical stage
Magnifications 60-1000X

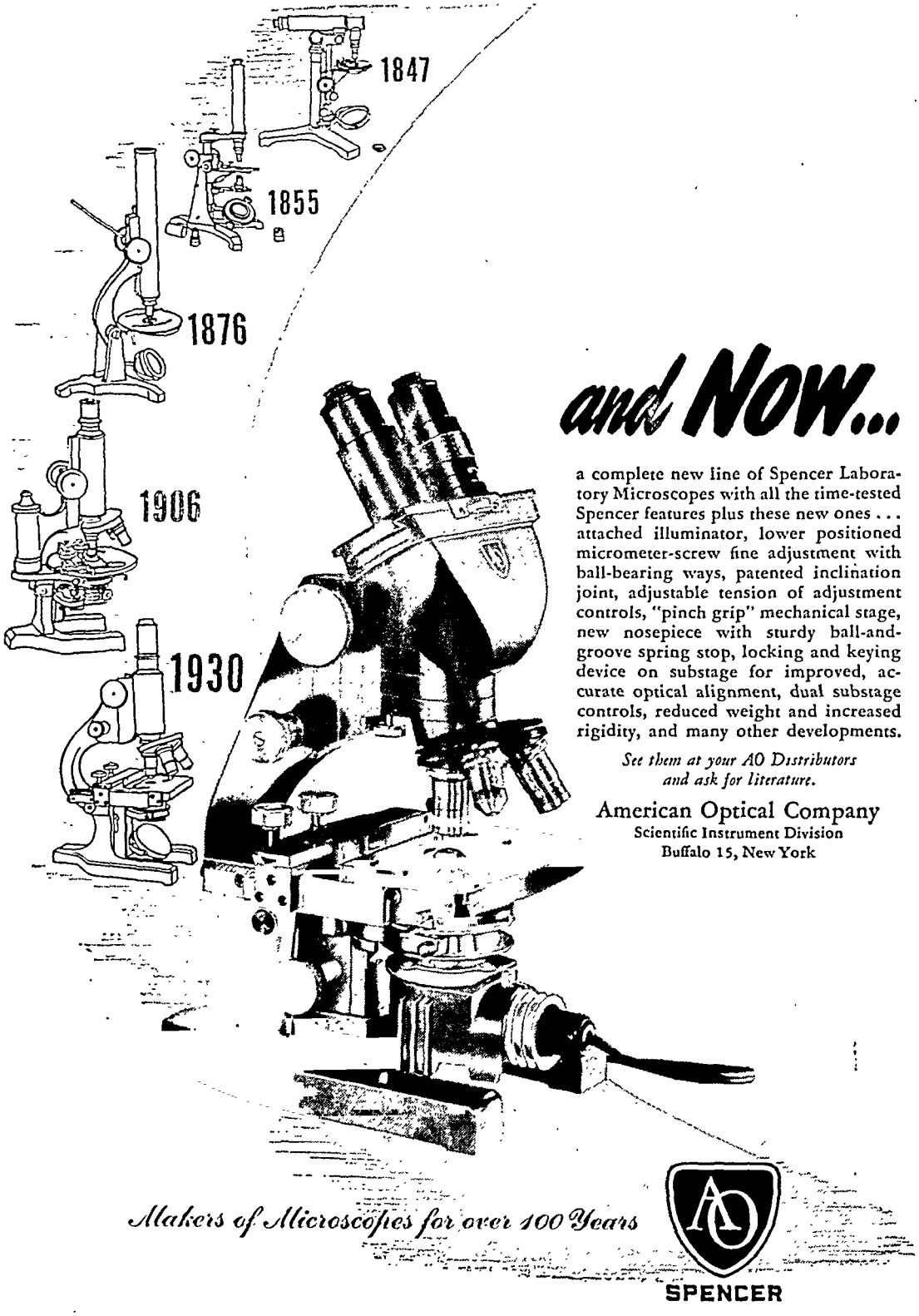
$298

Write today for Catalog Micro—100 B

E. LEITZ, Inc., 304 Hudson St., New York 13, N. Y.

LEITZ MICROSCOPES • BINOCULARS • SCIENTIFIC INSTRUMENTS
LEICA CAMERAS AND ACCESSORIES

(In writing to advertisers, please mention the Journal—it helps.)



and NOW...

a complete new line of Spencer Laboratory Microscopes with all the time-tested Spencer features plus these new ones . . . attached illuminator, lower positioned micrometer-screw fine adjustment with ball-bearing ways, patented inclination joint, adjustable tension of adjustment controls, "pinch grip" mechanical stage, new nosepiece with sturdy ball-and-groove spring stop, locking and keying device on substage for improved, accurate optical alignment, dual substage controls, reduced weight and increased rigidity, and many other developments.

See them at your AO Distributors and ask for literature.

American Optical Company
Scientific Instrument Division
Buffalo 15, New York

Makers of Microscopes for over 100 Years

SPENCER

we say they're better

and so will you when you've

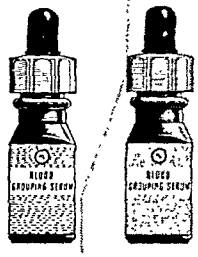
proved it in your own laboratory

HYLAND BLOOD GROUPING SERUM

Anti-A and Anti-B

Prepared from carefully selected donors to assure a highly potent, rapid acting, and specific product. Accurate results with maximum speed.

Available in 2 cc. and 5 cc. sets.



HYLAND ANTI-Rh SERUMS

Anti-Rh_0 (Anti-D) Typing Serum

Anti-Rh_0 rh' rh" (Anti-CDE) Typing Serum

Outstanding serum for slide method—rigidly tested for titer, avidity, specificity and sterility. Prepared from selected, immunized donors.

Both types available in 2 cc. and 5 cc. sizes.



make this test at our expense!

Clip the coupon for your free samples today! You'll receive generous samples of Rh_0 Typing Serum and Blood Grouping Serum. Enough to convince you that their speed, accuracy and titer are unsurpassed.

Hyland Laboratories

4534 Sunset Boulevard, Los Angeles 27, California

Please send me generous samples of Rh_0 and Blood Grouping Serums.

Your Name_____

Hospital or Lab. Name_____

Address_____

City and State_____

From what dealer do you regularly buy your typing serums?_____

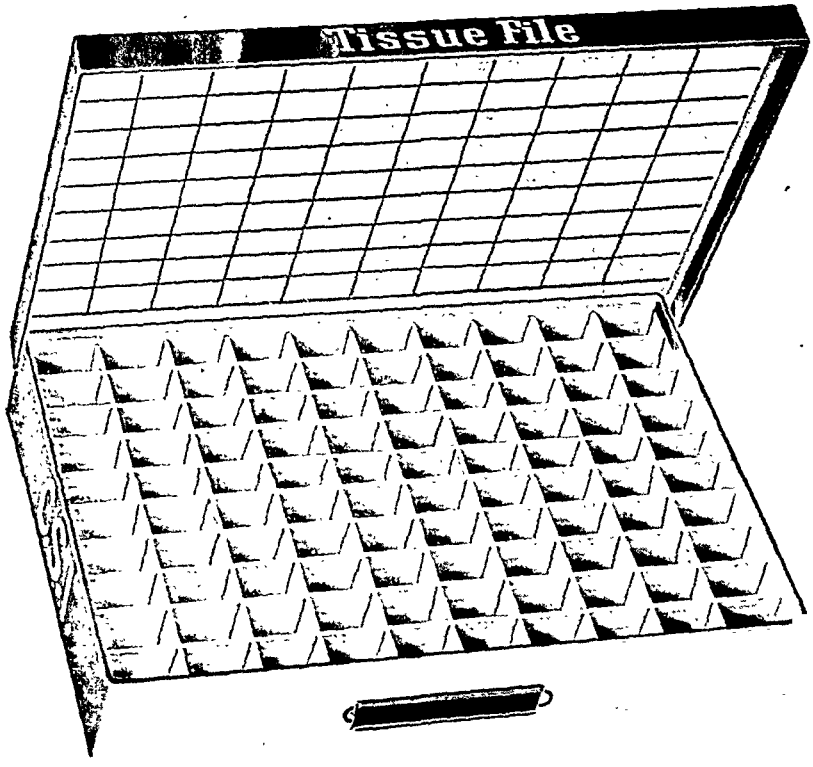
(In writing to advertisers, please mention the Journal—it helps.)

The Cargille

TISSUE FILE

For filing and storing Histology Tissues Embedded in Paraffin or Celloidin Blocks.

Will also prove indispensable in anatomy and other biological laboratories where tissues are studied by histological methods.

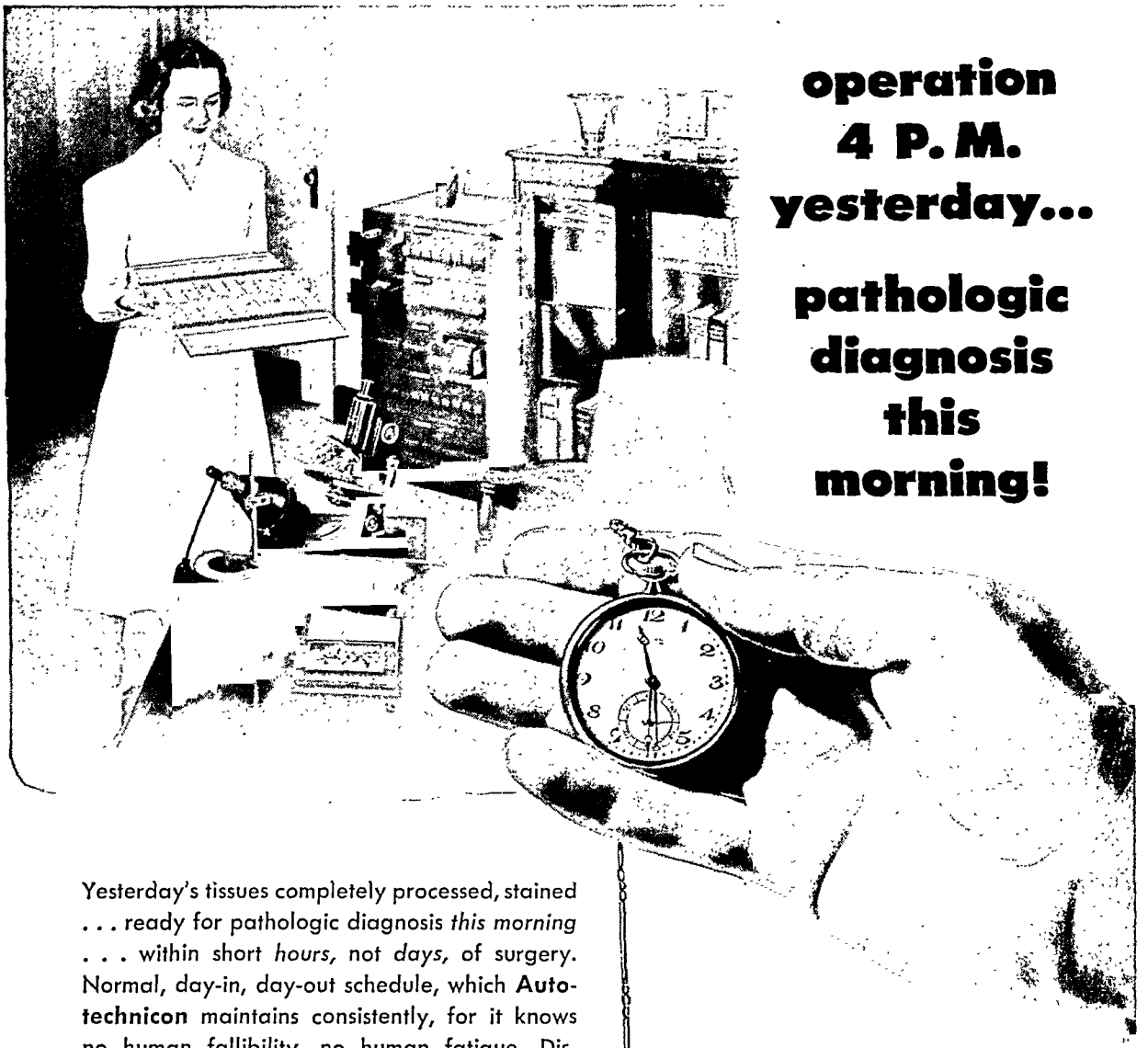


- Conveniently stores one hundred groups of blocks in one case. Each compartment will accomodate 10 to 15 blocks of the usual size.
- Removable partitions make it possible to double or quadruple the size of compartments.
- Indexing data (date, number, etc.) can be recorded on card in front of case.
- Inside of hinged cover is ruled into spaces corresponding to compartments. Data for indexing each lot of specimens can be recorded in proper space for instant reference.
- Sturdily constructed, specially reinforced. Designed so that each case can be conveniently numbered and stacked on shelves.

Case measures $15\frac{1}{4}'' \times 10\frac{1}{2}'' \times 2\frac{3}{4}''$ and is divided into 100 compartments, each measuring $1\frac{1}{2}'' \times 1'' \times 2\frac{1}{4}''$ deep. Shipping weight for each case $3\frac{1}{2}$ lbs. PRICE, 1 to 11 . . $2.75 ea; 1 doz. $27.50

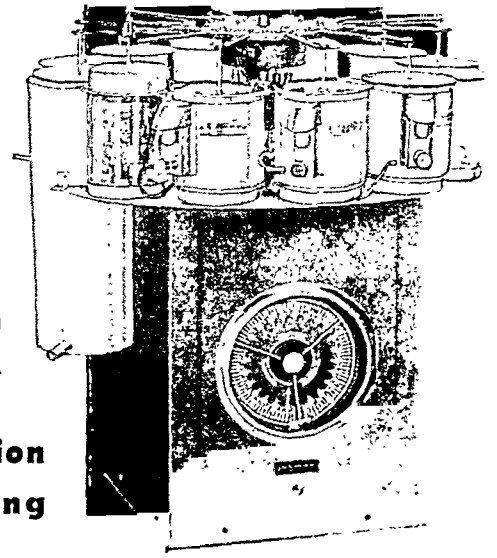
CARGILLE SCIENTIFIC INC.

118 LIBERTY STREET NEW YORK 6, N. Y.



operation 4 P.M. yesterday... pathologic diagnosis this morning!

Yesterday's tissues completely processed, stained . . . ready for pathologic diagnosis *this morning* . . . within short *hours,* not *days,* of surgery. Normal, day-in, day-out schedule, which **Autotechnicon** maintains consistently, for it knows no human fallibility, no human fatigue. Dispenses with tedious hand methods, with an overall gain in quality. The surgeon, as well as the pathologist, will find our brochure describing **Autotechnicon** of great interest. It is available on request.

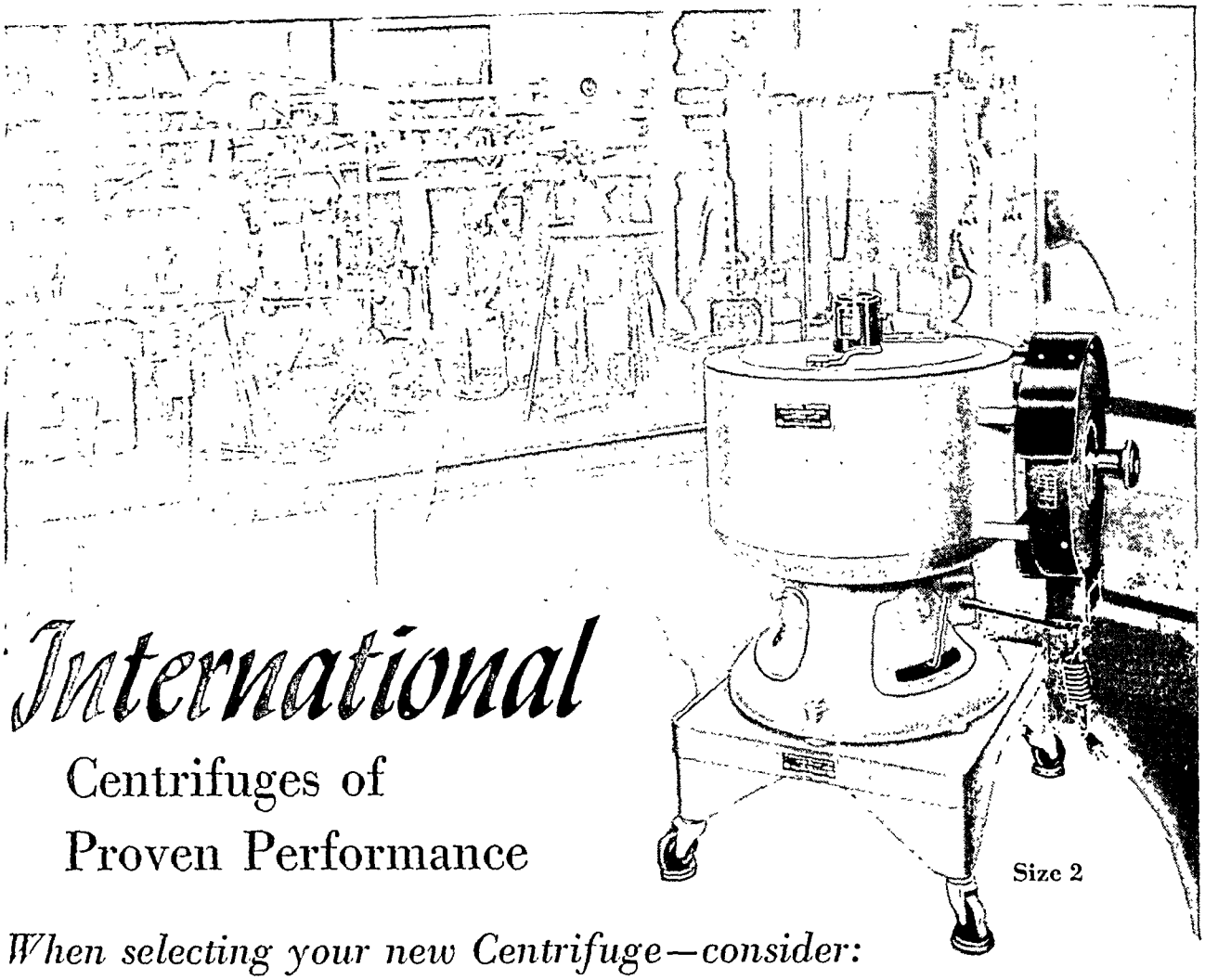


Autotechnicon

Trade Mark Registered U. S. Patent Office

automatic fixation, dehydration washing, infiltration, staining

THE TECHNICON COMPANY
215 East 149th Street • New York 51, N. Y.

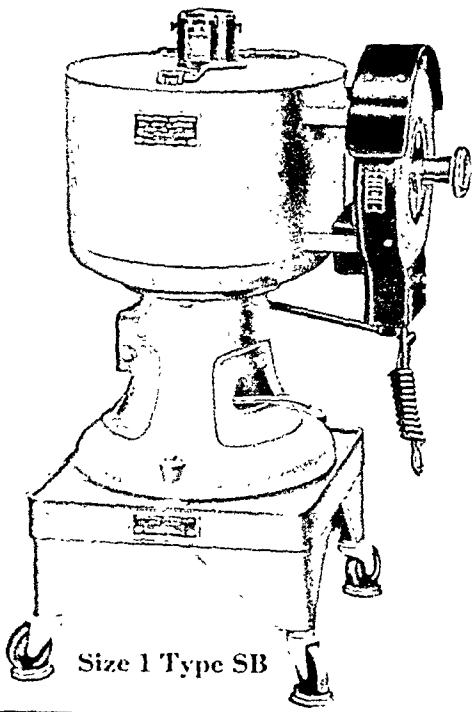


International

Centrifuges of Proven Performance

Size 2

When selecting your new Centrifuge—consider:



Size 1 Type SB

1 **The reliability of the electric motor that drives it.**
The motors of International Size 1 and Size 2 Centrifuges are specially designed to provide the best possible drive for a Laboratory Centrifuge. They are manufactured entirely in our own shops. thus eliminating the possibility of obsolescence through discontinuance by an "outside" manufacturer.

2 **The range of its application.**
Interchangeable accessories designed specifically for International Centrifuges are available in wide variety, making the machines adaptable to practically every laboratory application. Your future requirements as well as your present needs should be considered.

3 **The relation of first cost to total cost.**
Expensive repairs can make a low first cost a high cost in the long run. International Centrifuges are famous for their ability to give years of trouble-free service.

4 **The reputation of the manufacturer.**
International's fifty years of specialization in the manufacture of Laboratory Centrifuges is your guarantee of unequalled performance.

INTERNATIONAL EQUIPMENT COMPANY

1284 SOLDIERS FIELD ROAD, BOSTON 35, MASS.



NEW! ILLUMINATOR EXCLUSIVELY FOR MICROSCOPY

A NATURAL COMPLEMENT TO A GOOD MICROSCOPE

A good microscope requires a light source of equivalent quality if best possible results are to be obtained.

The new Illuminator is a specialized accessory for microscope use particularly valuable for binocular microscopes, both bright field and phase.

The Illuminator is also recommended for photomicrography, microprojection, dark field illumination, and work with both Kohler and parallel illumination. Some of its features are:

COOLER OPERATION • NO TRANSFORMER • PRECISE TILTING
PRECISION REFLECTOR ADJUSTMENT • RAPID BULB CHANGE
CRITICAL FOCUSING FROM EITHER SIDE • QUALITY OPTICS
SIMPLE FILTER HOLDER • IRIS DIAPHRAGM-KNURLED RING CONTROL

All adjustments are external and located for convenience. The ventilating fins on the aluminum body, coupled with the natural draft ventilation of the lamp chimney, assure cool operation.

11660-MICROSCOPE ILLUMINATOR, SPENCER No. 735B. With simple filter holder, iris diaphragm, Corning daylight glass, one 100 watt bulb, and 8 foot cord with switch $63.00

Write, Teletypewrite, Wire or Phone Dept. JCP-8 our Nearest Office-Warehouse

SERVING SCIENCE THROUGH SUPPLY

WILL CORPORATION
LABORATORY APPARATUS AND CHEMICALS
ROCHESTER 3, N. Y. • NEW YORK 12, N. Y.
SOUTHERN SCIENTIFIC CO., INC., ATLANTA 3, GA.
BUFFALO APPARATUS CORP., BUFFALO 5, N. Y.

THE PANRAY CORP.

pioneer manufacturer of DOPA

and other specialized chemicals

for investigation and research . . .

announces the availability of

Iodoacetic Acid "Panray"

(twice recrystallized, special reagent grade)

for the Iodoacetate Index Determination of Serum Proteins

Iodoacetic acid is a recently reported reagent for the determination of *deficient thermal coagulation of serum proteins* in cancer and other pathological conditions.

Originally reported by C. Huggins, M.D., G. M. Miller, M.D., and E. V. Jensen, Ph.D.: Thermal Coagulation of Serum Proteins—II. Deficient Coagulation in Cancer and the Iodoacetate Index —*Cancer Research, Vol. 9, No. 3, March 1949.*

DOPA "PANRAY"

(3,4-dihydroxyphenylalanine, C. P.)

available in l and dl forms

Synthetic chemical reagents . . . capable of revealing the presence of an oxidase in cells elaborating melanin. Valuable indicators of active melanogenesis.

Write for prices and other data

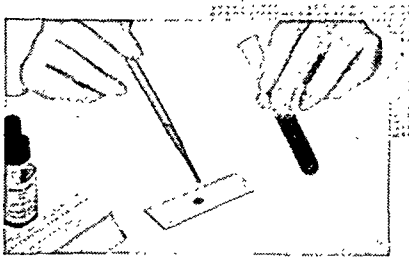
THE PANRAY CORP.

Custom Manufacturers of Fine Organic Chemicals

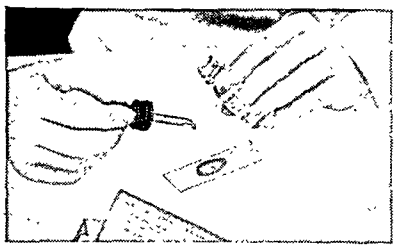
398 Broadway New York 13, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)

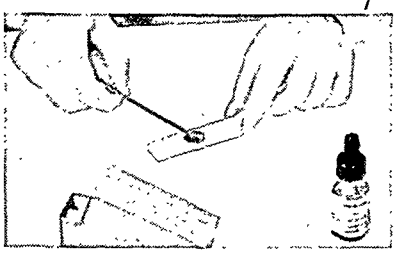
This Slide Method gives (Rh) test in seconds



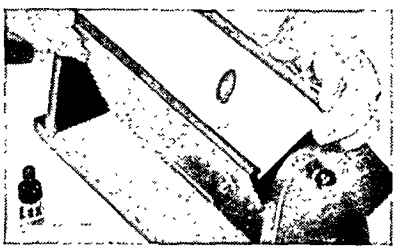
1 • *Place two drops of blood on slide. Fresh, clotted, oxalated, heparinized or citrated blood may be used.*



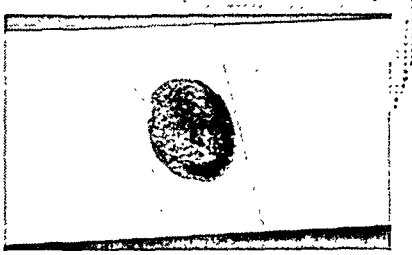
2 • *Add one drop of Anti-*Rh *serum.*



3 • *Mix Anti-*Rh *serum with blood and spread over slide.*



4 • *Place slide on view box. Tilt view box back and forth and watch for clumping. No microscope is needed.*



5 • *Clumping starts in 45 seconds, is complete in 1 to 2 minutes. Clumping indicates blood is* Rh *Positive; absence of clumping,* Rh *Negative.*

The Slide Method, using the view box, accounts in part for the rapidity and accuracy of Rh Tests. But even more important is the potency of the serums themselves.

Prepared under the strictest controls, and according to N. I. H. specifications, AMERICAN serums retain their original potency over a long period even under adverse conditions. Six strategically located distributing centers assure you of prompt service. Write today for complete descriptive literature.



PLAN WITH AMERICAN
... the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS
(In writing to advertisers, please mention the Journal—it helps.)



WATER SOLUBLE INDICATORS FOR pH DETERMINATIONS

The indicators listed below are now available as the sodium salts and are designated W.S. (water soluble). The older technique of using these indicators involved the use of insoluble indicators (free acid compounds) and effecting solution by the addition of definite amounts of standard sodium hydroxide solution. The use of the water-soluble indicator eliminates the difficulties encountered in the older procedure. The label upon the package carries information regarding the amount of the indicator to be used to prepare a solution corresponding to a 0.04% or 0.02% solution of the respective acid indicator.

NEW LOW PRICES

NAME	pH Range	1 gm.	10 gm.	25 gm.
Thymol Blue, W.S. (Thymol sulfon phthalein, sodium salt)	1.2–2.8 8.0–9.6	$1.20	$10.00	$22.50
Brom Phenol Blue, W.S. (Tetra bromo phenol sulfon phthalein, sodium salt)	3.0–4.6	1.20	10.00	22.50
Brom Cresol Green, W.S. (Tetra bromo-m-cresol sulfon phthalein, sodium salt)	4.0–5.6	2.40	20.00	45.00
Methyl Red, W.S. (Di methyl amino azo benzene-o-carboxylic acid, sodium salt)	4.4–6.0		.61	1.03
Brom Cresol Purple, W.S. (Di bromo ortho cresol sulfon phthalein, sodium salt)	5.2–6.8	1.20	10.00	22.50
Chlor Phenol Red, W.S. (Di chloro phenol sulfon phthalein, sodium salt)	5.0–6.0	2.40	20.00	45.00
Brom Thymol Blue, W.S. (Di bromo thymol sulfon phthalein, sodium salt)	6.0–7.6	1.20	10.00	22.50
Phenol Red, W.S. (Phenol sulfon phthalein, sodium salt)	6.8–8.4	1.20	10.00	22.50
Cresol Red, W.S. (Ortho cresol sulfon phthalein, sodium salt)	0.2–1.8 7.2–8.8	1.20	10.00	22.50
Meta Cresol Purple, W.S. (Meta cresol sulfon phthalein, sodium salt)	1.2–2.8 7.6–9.2	2.40	20.00	45.00

The COLEMAN & BELL Co., Inc.

Manufacturing Chemists : : Norwood, Ohio, U. S. A.

COLEMAN & BELL

(In writing to advertisers, please mention the Journal—it helps.)



"as handy a tool as I've ever seen"

the New, Improved

technicon paraffin knife*

will win you, too, once you curl your hand around it. It makes such light, fast work of so many laboratory chores dealing with paraffin-embedded tissues . . .

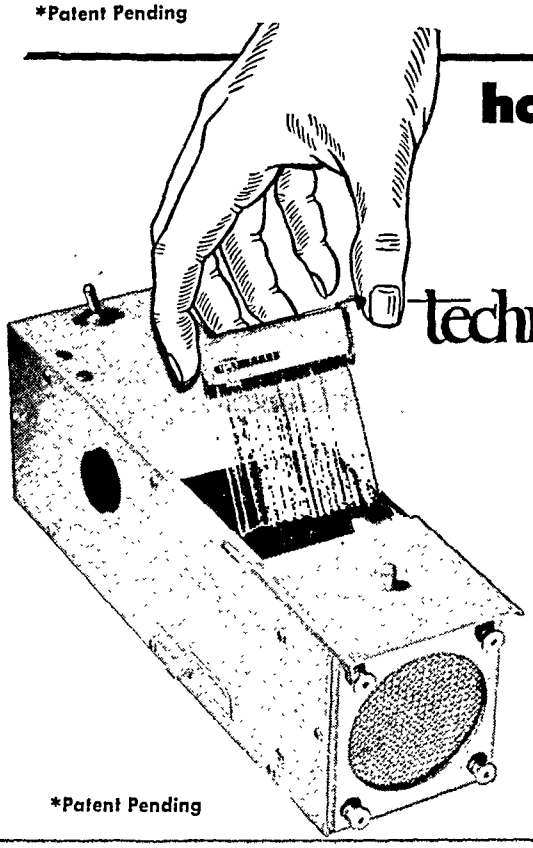
- **cutting large blocks into small;**
- **mounting blocks on a microtome object disc;**
- **squaring blocks to get even ribbons;**
- **separating ribbons in a water bath;**
- **sealing museum jars, etc.**

*Patent Pending

The hollow stainless steel handle fits your hand nicely; keeps cool during day-long use. The cord end and knife end are strong, moulded bakelite; the sharp-edged blade is cast beryllium-copper. The long-life nichrome heating element is housed within the hollow blade.

Finger-tip heating control is provided in an "on-off" spring switch under the thumb; easy to keep blade at just the right temperature. An ingenious saddle rest comes with the knife — lay knife in it **blade UP**, and switch is depressed, keeping blade hot; lay knife **blade DOWN**, and the current is "off."

Wgt. 12 oz. — 110 Volts AC-DC — 25 Watts



how to dry slides in 7 MINUTES

Just drop the batch, in an Autotechnicon Slide Carrier, into the hatch of *The New*

technicon microslide dryer*

flip the switch "on" and close the cover. A steady current of warm, filtered air blowing over them will carry away moisture through a duct in the side of the dryer. *In seven minutes your slides are ready for staining. That's all there is to it!*

They'll be clean and dust-free, as incoming air is filtered (filters replaceable). *No distortion* either — the warm air is well below the paraffin melting point. A blotter-lined sliding tray in floor catches drip — keeps inside clean.

Built of steel with durable baked-on gray enamel finish throughout. Size — 3½" x 4" x 12¼" long. Fits anywhere.

110 Volts AC — 75 Watts

*Patent Pending

technicon LAB-AID REQUISITES

THE TECHNICON COMPANY
215 East 149th Street, New York 51, N. Y.

Send me literature and prices on:

☐ The New, Improved Technicon Paraffin Knife
☐ The New Technicon Microslide Dryer

Name.....

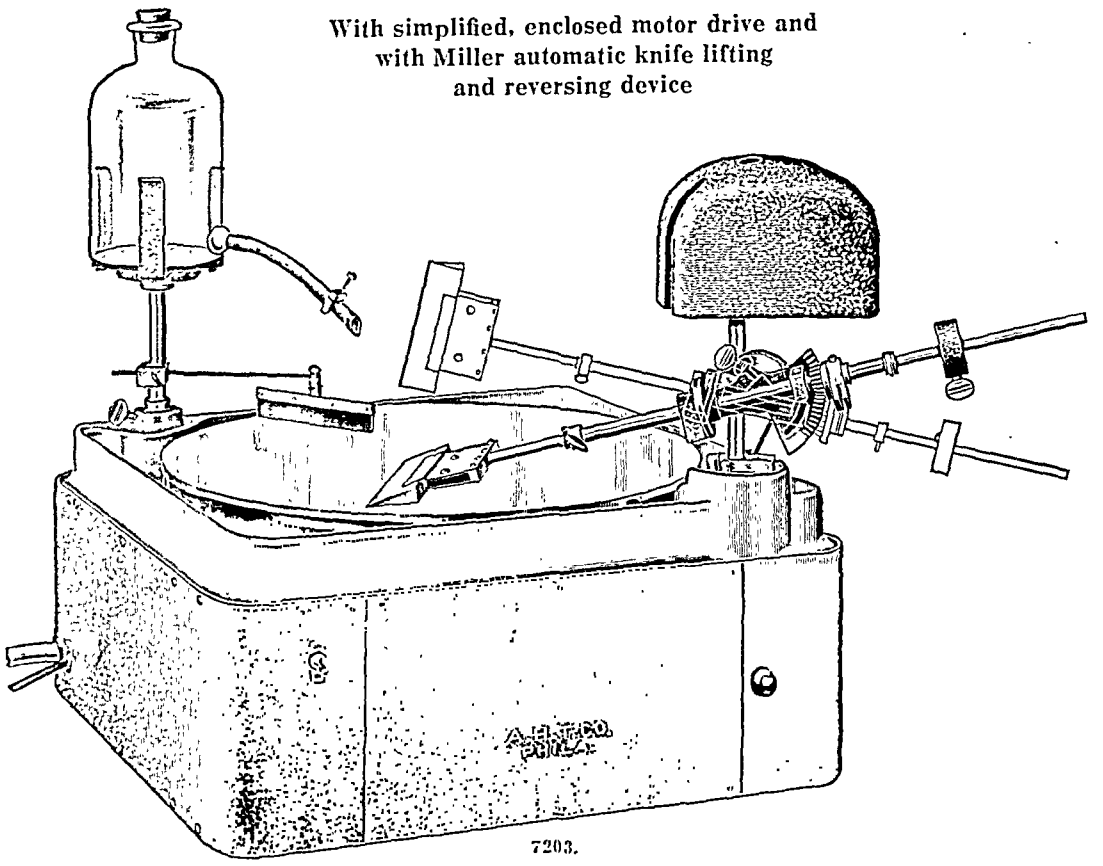
Street.....

City, Zone, State.....

IMPROVED MODEL, FANZ AUTOMATIC

MICROTOME KNIFE SHARPENER

With simplified, enclosed motor drive and
with Miller automatic knife lifting
and reversing device



7203.

MICROTOME KNIFE SHARPENER, FANZ AUTOMATIC, Improved Model. With simplified, enclosed motor drive and with Miller automatic knife lifting and reversing device. Takes knives up to 300 mm length and 12 mm thick. Provides more nearly perfect cutting edge than skillful hand honing and stropping.

A revolving glass disc, 21 inches diameter, is driven against the knife which is automatically swung to and fro in an arc through the center of the disc surface. The automatic attachment holds the knife in contact with the glass disc for 17.5 seconds, during which period it makes 5.5 reciprocating cycles, and is then lifted and turned over in 7 seconds and the process repeated on the opposite side. A scale on the automatic attachment provides for precise, reproducible setting of the angle of the bevel. Metal housing is 25 inches square x 11 inches high and is finished in gray crystal lacquer.

7203. **Microtome Knife Sharpener, Fanz Automatic, Improved Model,** as above described, with Miller knife turning and reversing device, complete with glass disc, drip deflector, glass reservoir, 2 liter capacity, rubber and Tygon tubing connections, and 1 lb. each of white rouge and castile soap. For 115 volts, 50 or 60 cycles, a.c... 576.00

More detailed information sent upon request.

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

Cable Address, "BALANCE," Philadelphia

THE ADHESIVENESS OF BLOOD PLATELETS IN THROMBOEMBOLISM AND HEMORRHAGIC DISORDERS

I. Measurement of Platelet Adhesiveness by the Glass-Wool Filter*

SYLVAN E. MOOLTEN, M.D., and LEO VROMAN

From the Research Division, Laboratory Service, St. Peter's General Hospital, New Brunswick, N. J.

One of the most pressing needs in the present broad offensive against thromboembolism is for a simple test by means of which the prethrombotic and early thrombotic state may be recognized in time for adequate prophylactic treatment. The number and variety of tests which have been proposed for this purpose reflect wide divergence of opinion on the underlying cause of thrombi and on the manner in which they form. The majority of these tests can be grouped in one of 2 categories, (1) tests of *blood coagulability* and (2) tests of *blood platelet form and behavior*. Although pathologic studies[5, 34, 35, 39] extending back for a century indicate that thrombosis begins as an occluding mass of sticky platelets (white thrombus) at a favored site and that coagulation occurs only secondarily in the trapped column of stagnant blood (red thrombus), the impression still prevails among a number of clinical observers that thrombosis is caused by increase in circulating thromboplastin and other elements concerned primarily in the coagulation mechanism. Coagulation time, as measured in whole blood[4, 16] or plasma[3, 26] or in diluted plasma with added thromboplastin[6, 31] ("prothrombin time"), has been reported variously as either accelerated[3, 4, 6, 16, 26, 31] or unchanged[13, 24, 29, 32] during active formation of a thrombus. Theoretically, increase in coagulability may be accounted for by rapid lysis of enormous numbers of platelets[20] which have agglutinated to form the white thrombus. On the other hand measurable depletion of prothrombin activity in the circulating blood may follow this phase of thrombosis, probably as the secondary clot (red thrombus) reaches massive proportions.[28] Reported discrepancies in results of the same tests in the hands of different observers are perhaps not necessarily attributable to faults in the methods themselves but to the fact that comparisons are made of individual readings instead of serial curves. In our own experience, the results of certain tests in the same patient may vary sharply with the stage of evolution of the thrombus.

RELATION OF PLATELETS TO THROMBUS FORMATION

The superiority of serial tests as a method of detection of thrombosis is well illustrated by the results of blood platelet studies. Our own data, for example, in confirmation of earlier observations of Kristenson,[18] Normann,[25] and Kojima,[17] indicate that formation of white thrombus can be suspected when the platelet

* Aided by grants from the Dazian Foundation, Dr. F. M. Clarke, and Mr. Harry R. Hillman.

Received for publication, May 5, 1949.

count drops sharply from previous normal or high levels, reflecting withdrawal from circulation of large numbers of platelets. Transient moderate drop in platelet count was noted quite regularly immediately after major surgical procedures, probably in measure with utilization of platelets in formation of multiple small wound thrombi as part of normal hemostasis.

Attempts to correlate increased platelet numbers with predisposition to thrombosis have fallen short of expectation[24, 36] despite the frequent association of the two in surgical convalescence and in the puerperium.[1, 10] Closer correlation has been reported to exist between alterations in blood platelet behavior and the tendency to thrombosis. Von Seemen and Binswanger[33] observed marked increase in platelet agglutinability three to six days after operation. To test this they employed the simple method described by Bürker[7] in 1903 in which a large drop of blood from the fingertip is allowed to stand in a concave depression on a small block of paraffin until a layer of plasma has separated at the summit of the drop, whereupon a coverslip is lightly applied to the latter, bringing away a droplet of plasma with its suspended platelets. The latter preparation, while be ng kept moist, is examined for the speed of platelet clumping and the size of clumps. Jürgens and Naumann[15] applied the identical principle to venous blood collected without anticoagulant in a paraffin-lined tube kept at refrigerator temperature during separation of the plasma. Øllgaard[27] devised a macroscopic method for measuring platelet agglutinability in terms of the minimal amount of mercuric chloride solution required to produce visible flocculation in citrated plasma previously exposed to a temperature of 42 C. for three hours to stabilize the platelet suspension. Copley[9] obtained stable platelet suspensions merely by thoroughly washing and resuspending platelets in physiologic saline, and tested their agglutination upon addition of various samples of plasma or serum or fractions of the latter; results of clinical application of his method in detection of thromboembolic tendencies have not yet been published.

Platelet adhesiveness to a glass surface was measured by Morawitz and Jürgens[23] in terms of minimal plugging time of a glass capillary tube in which a sample of blood is forced back and forth by means of a mechanical pump, the "capillary thrombometer." A simpler and more reliable method was later devised by Wright,[37] in which heparinized blood is placed in a specially constructed paraffin-lined glass tube with central bulb whose sides have been carefully ground away in 2 places to form open windows about 1.5 cm. in diameter. These windows are then closed by cover glasses kept in place by Vaseline but otherwise uncoated. The tube is rotated at three and one-half r.p.m. on a motor-driven wheel so that blood passes constantly over a known area of uncoated glass (about 33 sq. mm.) at a steady rate. Platelet counts are made on samples withdrawn every 20 minutes for a period of 80 minutes, and expressed as percentages of the initial count. The long test period of Wright's method makes it theoretically likely that some of the platelet loss results from lysis as well as adhesion to the glass (Baar[2]). Inasmuch as lysis of platelets is probably initiated by thrombin,[30] the results of the test in hemophilia, hypoprothrombinemia, and other conditions in which thrombin production is much retarded, may reflect diminished rates of platelet lysis and not necessarily diminished platelet adhesiveness.

PLATELET ADHESIVENESS TO "WETTABLE" AND "NONWETTABLE" SURFACES

The lack of correlation between platelet lysis and platelet adhesiveness *in vitro* finds its parallel in the lack of correlation between blood clotting and platelets in respect to foreign surfaces. Although clotting of whole blood or plasma can be greatly retarded if the specimens are collected in receptacles coated with paraffin, collodion, plastic materials (*e.g.*, lusteroid), and silicone rather than in uncoated glass or other "wettable" surface,[14, 19] platelets can be readily shown to adhere indiscriminately to both wettable and nonwettable surfaces in more or less equal degree. Thus in one experiment an ordinary glass slide was marked off into equal segments, one of which was left unchanged, and the others coated

TABLE 1

COMPARATIVE ADHESIVENESS OF BLOOD PLATELETS TO WETTABLE AND NONWETTABLE SURFACES

TYPE OF BLOOD SAMPLE	NUMBER OF ADHERENT PLATELETS IN EQUAL UNIT AREAS OF SLIDE				
	Wettable Surface	Nonwettable Surfaces			
	Uncoated Glass	a) Paraffin Coating	b) Collodion Coating	c) Silicone Coating	d) Silicone Coating Treated with Alkali, then "Cleaning Solution"
Direct from vein of normal subject (L.V.)	86	—	108	119	109
	103	—	127	165	192
Citrated, from normal subject (G.M.V.)	124	120	120	123	147
Direct from vein of patient with polycythemia vera (W.R.)	573	442	500	584	488
	NUMBER OF MACROSCOPICALLY VISIBLE PLATELET THROMBI				
	15	11	7	2	8

respectively with (a) paraffin, (b) collodion, (c) silicone and (d) silicone followed by alkali, "cleaning solution" (potassium bichromate-sulfuric acid solution) and water. Blood was flooded over the surface of a few such slides directly from a #22-gauge needle inserted into a subject's vein or from a silicone-coated syringe in which the blood had been mixed with sodium citrate. After 30 seconds or longer the blood was rinsed off gently in saline until all red color was gone, the slides were fixed and stained, and the platelets were counted in equal areas of each segment. As seen in Table 1, the differences were not at all striking and probably lay within the range of experimental error.

In another experiment, citrated blood taken from a patient (Table 1) with polycythemia vera was passed repeatedly through a glass tube coated with silicone except over one small bare area. Many small platelet thrombi with minute tags of fibrin were deposited throughout the tube *except* upon the bare

area of glass. Apparently the adhesion of platelets to nonvital surfaces is governed by different principles from those concerned in the case of living blood vessels. A possible clue to the difference lies in the fact that silicone, paraffin, and similar materials form coatings nonwettable to blood or water yet wettable to oily substances. According to the conception put forward by Clowes[8] (1916) the surface of living cells represents a boundary between oil-in-water and water-in-oil emulsions in an equilibrium with each other determined by a critical ratio of sodium and calcium ions. Theoretically, therefore, the behavior of blood platelets in contact with foreign surfaces may resemble that of either water or oil droplets depending upon the circumstances. In this respect it is noteworthy that human platelets have been shown to possess a total lipid content of 16 per cent (Erickson *et al.*[11]).

For practical purposes, therefore, the nature of the foreign test surface used to measure platelet adhesiveness can be regarded as much less important than the quality of the protoplasmic surface of the platelets themselves. Their extreme lability, particularly in the presence of even minimal amounts of thrombin,[12] emphasizes the importance of careful control of *in vitro* conditions and, wherever possible, the use of short test periods.

By the use of a wick of braided glass-wool instead of a smooth glass surface, the authors have devised a method in which a relatively small quantity of citrated blood is exposed to a maximal surface of glass for a very short time so that the factor of platelet lysis is probably obviated to a large extent. Although intended originally as a means of assaying the activity of certain biologic agents (thrombocytopen, thrombocytosin[21]) through their effect on platelets *in vitro*, the method soon proved a useful supplement of the platelet count in studies of a variety of clinical conditions affecting the continuity of blood flow.

METHOD

1. *Preparation of glass-wool filter.* Commercial glass-wool of good grade* is drawn into 3 strands of equal size and worked into a loose braid 0.5–1.0 cm. in thickness of convenient length. Several such braids are prepared at one time, washed thoroughly in acetone, and kept in a covered jar. In preparation of a filter a segment of braid is cut 6.0–7.0 cm. in length and split a short distance at one end to provide 3 supporting arms by which it is suspended vertically within a silicone-coated (or paraffin-coated) test-tube of 2.0 cm. internal diameter. The arms of the braid are secured over the rim of the tube by an elastic band (Fig. 1). Loss of blood into the supporting arms through wick action is prevented by saturating them with melted paraffin, care being taken to keep the braid itself free. The filter is readied for use by moistening it with 1.5 ml. of physio-

* *Fiberglas,* Catalogue No. 800, Pyrex Brand Wool Filtering Fibre, distributed by Corning Glass Works, Corning, N. Y. A strand of standard thickness may be prepared, if desired, from ESE 6.2 sliver, procurable from the same firm. The sliver is washed in five changes of hot acetone to remove lubricant, and dried. Four such slivers together form a strand of suitable thickness for braiding.

A prepared filter has been devised recently, consisting of a small plastic funnel into the lower part of which the appropriate length of glass-wool braid is secured. Additional details may be secured from the authors.

logic saline, covering the upper end with a loose-fitting bakelite cap to prevent evaporation, and warming it in an incubator to 37 C.

2. *Collection of blood sample.* Through a #20–#23 gauge needle 1.8 ml. of blood is drawn quickly from the subject's vein into a 3 ml. (or 5 ml.) syringe (preferably coated with silicone*) containing 0.2 ml. of 3.8 per cent sodium citrate solution. The syringe is inverted 3 or 4 times and the blood it transferred to a paraffin-coated (or silicone-coated) collecting tube of about 1.5 cm. internal diameter.

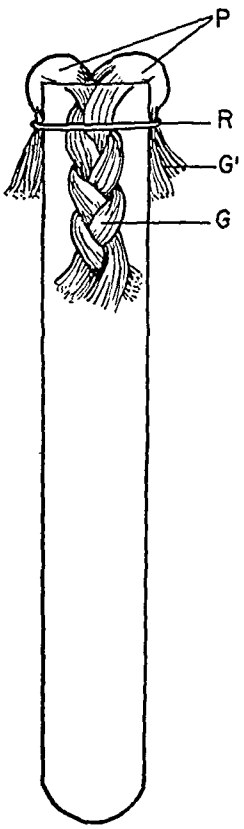


FIG. 1. Diagram of prepared filter: G, glass-wool braid suspended in test tube by supporting arms G' (single strand on left, double strand on right); P, paraffin coating to prevent loss of blood into supporting arms; R, elastic band securing latter over rim of test tube.

3. *Pre-filtration platelet count.* Blood is drawn immediately from the collecting tube into a *red cell* counting pipet to the 0.5 mark and diluted to the 101 mark with 3.8 per cent sodium citrate solution containing 0.2 per cent formalin and shaken in a pipet-shaker at about 200 times a minute for 10 minutes.

4. *Filtration.* Exactly 10 minutes after the moment of drawing of the blood sample from the subject's vein the collecting tube is gently agitated and 1.0 ml. of blood is taken from it into a volumetric pipet. The prepared filter, withdrawn from the incubator immediately before this, is uncapped, and the blood in the pipet is permitted to drip into the glass-wool as quickly as can be accomplished without overflow or leakage outside the confines of the braid. The latter should now appear distended with blood but only a drop or two of clear saline should escape from its lower end. Exactly 30 seconds later the blood in the braid is washed through with 8 ml. of chilled eluting fluid, consisting of 1 part of 3.8 per cent sodium citrate solution added to 9 parts of physiologic saline and cooled before use to about 5 C. To avoid overflow or leakage it is added to the filter drop by drop during a period of one and one-half to two minutes. The washed braid is then removed (without squeezing) and discarded.

5. *Post-filtration platelet count.* The filtrate is mixed well by inverting the test tube 5 times. A sample is then drawn into a *white cell* counting pipet to the 0.5 mark and diluted to the 11 mark with the formalinized citrate solution used in (3) and placed in the pipet-shaker for 10 minutes.

6. *Computations.* Upon completion of the required period of pipet-shaking, standard hemocytometer chambers are filled with the respective cell suspensions (3, 5) and permitted to stand 10 minutes on a level surface for settling. Evaporation is prevented by covering the chambers with a petri dish lined on its flat inner surface with moist filter paper. Counts are made of the red blood cells of 5 small squares

* According to directions accompanying material (G. E. *Drifilm* 9987)

and of the platelets of 25 small squares (1 large square). When their number appears significantly less than normal the platelets are enumerated in 5 large squares and the total is divided by 5. The data are charted as follows:

	A. UNFILTERED	B. FILTERED
1. RBC/5 small squares	440*	466
2. Platelets/25 small squares × 2	188	184
3. Total RBC (corrected for added citrate)	440 × 10/9 × 10,000 = 4,890,000	
4. Total platelets (corrected for added citrate)	188 × 10/9 × 1000 = 209,000	
5. RBC: platelet ratio (computed from #1 and #2)	23.4	25.3
6. *Adhesive index* (5B/5A)	1.08	

Empirically we have found that in subjects with presumably "zero" platelet adhesiveness (*e.g.*, thrombocytopenic purpura secondary to hypersplenism) the adhesive index approaches or reaches 0.7 as its base-line rather than the theoretical value of 1.0, indicating that the filter is more permeable for platelets than for erythrocytes under such circumstances. The adhesiveness of erythrocytes, measured by computation from pre- and post-filtration counts, fluctuated within a narrow range numerically which was too small in general to be of practical significance. The sole exception was polycythemia vera in which a significant loss of erythrocytes occurred in the filter, correlated with greatly increased viscosity of the whole blood.

If the adhesive index 0.7 is taken as representing "zero" adhesiveness, a numerical approximation of the number of adhesive platelets may be made by the following calculations:

$$\text{Nonadhesive platelets} = \frac{\text{total platelets}}{\text{adhesive index} + 0.3}$$

$$\text{Adhesive platelets} = \text{Total platelets} - \text{nonadhesive platelets}$$

$$= \text{Total platelets} \times \left(1 - \frac{1}{\text{adhesive index} + 0.3}\right)$$

In the example given above, the total platelet count is 209,000, adhesive index 1.08. The adhesive platelet count $= 209{,}000 \times \left(1 - \frac{1}{1.38}\right) = 58{,}000$†

DISCUSSION

Although simple in principle, the filter test gave consistent results only when performed in strict conformity with the steps outlined. For example, the interval between withdrawal and filtration of the blood sample was standardized at 10 minutes to allow for collection of specimens in various parts of the hospital or nearby locations. Deviations led to considerable discrepancy in results, as

* Data from a representative subject.

† Actually such a figure probably represents an approximation of the *stickier* platelets inasmuch as all platelets theoretically possess a certain amount of stickiness, which varies in accordance with test conditions and other extrinsic factors.

demonstrated in 3 experiments in which the interval was deliberately lengthened. In one experiment the adhesive index rose from 0.76 to 2.21 in blood permitted to stand 67 minutes longer at room temperature, in another experiment from 1.33 to 2.93 after 63 minutes, whereas in a third experiment the index which was 1.34 at 10 minutes was found to be 1.25 after 48 minutes. Such changes in adhesiveness were retarded in varying degree by refrigeration of the sample at 4 C. and in some instances the index dropped even below the control level. These results are of more than incidental interest for the light they shed on individual differences, also on the desirability of short rather than long *in vitro* tests of platelets.

The special nature of platelet adhesiveness, including its independence of the plasmatic factors which affect the sedimentation rate of erythrocytes, was suggested by Wright.[38] Comparative tests of erythrocyte sedimentation rate and platelet adhesiveness in our hands led us to similar conclusions. In addition no correlation was observed between platelet adhesiveness and erythrocyte adhesiveness when, as indicated above, both were determined together from the same filter adsorption figures. Except in the case of polycythemia vera, in which blood viscosity also proved much higher than normal, erythrocyte adhesiveness by this method was found to be more or less constant regardless of the underlying clinical condition.

The results of several hundred experiences with the filter test have demonstrated its possibilities as a useful supplement to the platelet count in evaluating the nature and progress of the thrombotic state and of various hemorrhagic disorders.[22] Normal platelet adhesiveness was found to lie within fairly narrow limits. The normal adhesive index, for example, ranged between 1.0 and 1.4. The adhesive platelet count, computed from the adhesive index and total count by the formula given above, ranged normally between 60,000 and 110,000. In individual cases examined repeatedly less fluctuation was found in the latter than in the adhesive index itself or in the total count which normally lay between 200,000 and 350,000.

SUMMARY

A method is described in which a wick of braided glass-wool is employed as an adsorbing filter for separation of adhesive platelets from nonadhesive platelets in citrated blood, making possible an enumeration of their relative proportions in the total platelet count. The relatively large surface area and short period of contact between the blood sample and the glass-wool fibers are believed to obviate to a large extent the possible error which may arise from lysis of platelets in methods involving prolonged test periods *in vitro*. The suggestion is made that the time factor in the latter type of test is likely to result in erroneously low findings of platelet adhesiveness in such conditions as hemophilia and hypoprothrombinemia in which platelet lysis is ordinarily retarded *in vitro* because of delayed or reduced thrombin formation. The normal range of adhesive platelet count has been found to lie between 60,000 and 110,000 platelets per cu. mm. and the adhesive platelet count in particular normal individuals

has been seen to vary less from time to time than the total platelet count. Platelet adhesiveness appears to be independent of the adhesiveness or sedimentation rate of erythrocytes. It is manifested impartially *in vitro* on wettable and non-wettable surfaces alike. The glass-wool filter method of measuring platelet adhesiveness is relatively simple and can be readily applied clinically as an aid to detection of predisposition to thrombosis and related conditions.

REFERENCES

1. Adams, E.: Postoperative thrombocytosis. Arch. Int. Med., **73**: 329–335, 1944.
2. Baar, H.: The stickiness of platelets. Lancet, **2**: 775, 1941.
3. Bancroft, F. W., Stanley-Brown, M., and Chargaff, E.: Postoperative thrombosis and embolism. Ann. Surg., **106**: 868–879, 1937.
4. Bergquist, G.: Über postoperative Thrombosen. Acta chir. Scandinav., **83**: 415–434, 1940.
5. Bizzozero, J.: Ueber einen neuen Formbestandtheil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. Virchows Arch. f. path. Anat., **90**: 261–332, 1882.
6. Brambel, C. E., and Loker, F. F.: Significance of variations of prothrombin activity of dilute plasma. Proc. Soc. Exper. Biol. and Med., **53**: 218–220, 1943.
7. Bürker, K.: Eine einfache Methode zur Gewinnung von Blutplättchen. Centralbl. f. Physiol., **17**: 137–138, 1903.
8. Clowes, G. H. A., cited by Jacobs, M. H.: Permeability of the Cell to Diffusing Substances. In Cowdry, E. V.: General Cytology. Chicago: The Univ. of Chicago Press, Section III, p. 155, 1924.
9. Copley, A. L., and Houlihan, R. B.: On the mechanism of platelet agglutination. Federation Proc., **4**: 173, 1945.
10. Dawbarn, R. Y., Earlam, F., and Howel Evans, W.: The relation of the blood platelets to thrombosis after operation and parturition. J. Path. and Bact., **31**: 833–873, 1928.
11. Erickson, B. N., Williams, H. H., Avrin, L., and Lee, P.: The lipid distribution of human platelets in health and disease. J. Clin. Investigation, **18**: 81–85, 1939.
12. Fonio, A., and Schwendener, J.: Die Thrombozyten des menschlichen Blutes, und ihre Beziehung zum Gerinnungs- und Thrombosevorgang. Bern, Switzerland: Hans Huber, p. 130, 1942.
13. Hurn, M., Barker, N. W., and Mann, F. D., Variations in prothrombin and antithrombin in patients with thrombosing tendencies. Am. J. Clin. Path., **17**: 709–711, 1947.
14. Jaques, L. B., Fidlar, E., Feldsted, E. T., and Macdonald, A. G.: Silicones and blood coagulation. Canad. M. A. J., **55**: 26–31, 1946.
15. Jürgens, R., and Naumann, W.: Klinische und experimentelle Untersuchungen über Funktionen der Blutplättchen. Deutsches Arch. f. klin. Med., **172**: 248–259, 1931.
16. Kadish, A. H.: Coagulation of the blood in lusteroid tubes; a study of normal persons and patients with arterial or venous thrombosis. Am. Heart. J., **34**: 212–224, 1947.
17. Kojima, S.: Experimentelle Untersuchungen über Veränderungen des Blutes durch aseptische Thrombenbildung. Arch. f. klin. Chir., **174**: 216–241, 1933.
18. Kristenson, A.: Beobachtungen über die Thrombozytenzahl bei klinischer Venenthrombose. Acta med. Scandinav., **69**: 453–481, 1928.
19. Lozner, E. L., Taylor, F. N. L., and MacDonald, H.: The effect of foreign surfaces on blood coagulation. J. Clin Investigation, **21**: 241–245, 1942.
20. Milstone, J. H.: Activation of prothrombin by platelets plus globulin. Proc. Soc. Exper. Biol. and Med., **68**: 225-228, 1948.
21. Moolten, S. E.: Studies on extractable factors in the spleen and other organic sources which influence the blood platelet count. J. Mt. Sinai Hosp., **12**: 866–873, 1945.
22. Moolten, S. E., Vroman, L., and Vroman, G. M. S.: The adhesiveness of blood platelets in thromboembolism and hemorrhagic disorders; diagnostic and prognostic significance of platelet adhesiveness. Am. J. Clin. Path., in press.
23. Morawitz, P., and Jürgens, R.: Gibt es eine Thrombasthenie? München. med Wchnschr., **47**: 2001, 1930. Cited by Morawitz, P.: Thrombose. Verhandl. d. deutsch. Ges. f. Kreislauff., **7**: 80–101, 1934.
24. Moses, C.: The effect of digitalis, epinephrine, and surgery on the response to heparin. J. Lab. and Clin. Med., **30**: 603–607, 1945.
25. Normann, E.: Wie verhalten sich die Thrombocyten nach operativ behandelten Krankheitsfällen und bei der Entstehung postoperativer Thrombose? Deutsche Zstchr. f. Chir., **212**: 166–178, 1928.
26. Nygaard, K. K., and Brown, G. E.: Essential thrombophilia; report of 5 cases. Arch. Int. Med., **59**: 82–106, 1937.

27. ØLLGAARD, E.: On the agglutination of the blood platelets under normal and pathological conditions. Acta med. Scandinav., **115:** 1–21, 1943.
28. Personal observations (unpublished).
29. POTTS, W. J., AND PEARL, E.: A study of the platelet count and the coagulation time of plasma and whole blood following operation. Surg., Gynec., and Obst., **731:** 492–494, 1941.
30. QUICK, A. J.: Studies on the enigma of the hemostatic dysfunction of hemophilia. Am. J. M. Sc., **214:** 272–279, 1947.
31. SHAPIRO, S.: Hyperprothrombinemia, a premonitory sign of thromboembolization (description of a method). Exper. Med. and Surg., **2:** 103–109, 1944.
32. TUFT, H. S., AND ROSENFIELD, R. E.: Significance of the accelerated reaction in determination of prothrombin time of diluted plasma. Am. J. Clin. Path., **17:** 704–708, 1947.
33. VON SEEMEN, H., AND BINSWANGER, H.: Uber Allgemeinveränderungen, besonders des Blutes, nach chirurgischen Eingriffen und ihre Bedeutung für Entstehung und Bekämpfung der mittelbaren Operationsschädigungen. Deutsche Ztschr. f. Chir., **209:** 157–210. 1928.
34. WELCH, W. H.: Thrombosis. In Allbutt's System of Medicine. London: Macmillan Co., **7:** 155–228, 1899. Reprinted in Papers and Addresses. Baltimore: Johns Hopkins Press, **1:** 110–192, 1920.
35. WHARTON JONES, T.: On the state of the blood and the blood-vessels in inflammation, ascertained by experiments, injections, and observations by the microscope (the "Astley-Cooper" prize essay for 1850). Guy's Hospital Rep., **7:** 1–101, 1851.
36. WINDFELD, P.: Beiträge zur Kenntnis der postoperativen Blutveränderungen. Acta chir. Scandinav., **70** (Suppl. 25): 9–137, 1933.
37. WRIGHT, H. P.: The adhesiveness of blood platelets in normal subjects with varying concentrations of anti-coagulants. J. Path. and Bact., **53:** 255–262, 1941.
38. WRIGHT, H. P.: Changes in the adhesiveness of blood platelets following parturition and surgical operations. J. Path and Bact., **54:** 461–468, 1942.
39. ZAHN, F. W.: Untersuchungen über Thrombose, Bildung der Thromben. Virchows Arch. f. path. Anat., **62:** 81–124, 1875.

QUANTITATIVE CORRELATION OF MORPHOLOGIC LIVER CHANGES AND CLINICAL TESTS*

HANS POPPER, M.D., FREDERICK STEIGMANN, M.D., AND PAUL B. SZANTO, M.D.

From The Hektoen Institute for Medical Research and Departments of Pathology and Internal Medicine of Cook County Hospital; Department of Pathology, Northwestern University Medical School; and Department of Internal Medicine, University of Illinois College of Medicine, Chicago, Illinois

The correlation between structural changes of the liver and clinical and laboratory findings is still undetermined. This is evident from a comparison of the results of the hepatic tests with the histologic picture of the liver as seen in biopsies.[3, 11, 22, 31, 32, 37] Even if there should be no correlation, in individual cases, between the results of the hepatic tests and the morphologic picture, it appeared worthwhile to subject a large material to statistical analysis. In a previous study,[7] statistically significant correlations were found between the presence of some morphologic changes and abnormal results of some of the hepatic tests, without reference to the degree of the abnormality of either the morphologic or the functional findings. The present study deals with an attempt to correlate statistically, on a larger sample than was previously done, the degree of aberration in the results of the hepatic tests with the severity of the liver cellular changes. The study was undertaken, (1) to determine whether a statistical relationship can be demonstrated between abnormalities in the results of the hepatic tests and the degree of liver cellular changes; (2) to determine which hepatic tests (if any) show the best correlation and, therefore, best reflect changes in the liver cells; (3) to establish if the number of tests with abnormal results is related to the degree of liver cell damage; (4) to find out if correlation between the severity of liver cell change and the degree of functional impairment is related to the specific liver disease present; and finally (5) to select the most promising tests that may be used as screening tests for the presence of liver cell damage.

MATERIAL AND METHODS

From a total of 198 patients, 257 specimens of tissue from the liver were available for biopsy; 196 specimens were obtained by aspiration (with the Turkel needle[36]), and 61 by surgical excision during a laparotomy performed for a variety of upper abdominal conditions. At the time of, or at most within two days before or after obtaining the specimen, a series of hepatic tests was performed which are listed in Table 1. The range of abnormality was arbitrarily graded from 0 to 3+. In the grading, abnormal elevation of total serum protein or abnormal reduction of the serum cholesterol (as might occur in severe liver

* Supported by grants from the Jerome D. Solomon Memorial Research Foundation and the Committee on Scientific Research of the American Medical Association.

Read by title at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, May 6, 1949.

damage) were not considered since they did not occur in this series; neither were abnormal reduction or absence of urinary urobilinogen considered, since it

TABLE 1

RESULTS OF HEPATIC TESTS PERFORMED SIMULTANEOUSLY WITH REMOVAL OF TISSUE FROM LIVER FOR BIOPSY

TEST	METHOD USED	DEGREE OF ABNORMALITY			
		0	+	++	+++
Cephalin cholesterol flocculation	Hanger[10]	0,-,+	++	+++	++++
Thymol turbidity, units	Maclagan,[18] Calibration according to Shank and Hoagland[30]	below 4.0	4.1–8.0	8.1–15.0	above 15.0
Total protein, Gm./100 ml. serum	Hoffman[12]	above 6.0	6.0–5.5	5.4–4.5	below 4.5
Albumin, Gm./100 ml. serum	Howe[13]	above 3.8	3.2–3.8	3.1–2.8	below 2.8
Globulin, Gm./100 ml. serum	Howe[13]	below 2	2.0–2.5	2.6–3.0	above 3.0
Albumin/globulin ratio	Howe[13]	above 1.25	1.25–1.1	1.05–0.9	below 0.9
Nonprotein nitrogen, mg./100 ml. serum	Hoffman[12]	below 35	35–50	51–70	above 70
Total cholesterol, mg./100 ml. serum	Bloor[1]	below 200	200–300	301–400	above 400
Total serum bilirubin, mg./100 ml. serum	Watson and Ducci[39]	below 1.2	1.2–4.0	4.1–10.0	above 10.0
1 minute (direct) serum bilirubin, mg./100 ml. serum	Watson and Ducci[39]	below 0.4	0.4–1.0	1.1–5.0	above 5.0
Prothrombin time, per cent	Quick[27]	above 85	85–70	69–60	below 60
Serum alkaline phosphatase, Bodansky units	Bodansky[2]	below 4	4.1–8.0	8.1–15.0	above 15
Urinary urobilinogen, Ehrlich units	Watson *et al.*[41]	below 3	3–5	6–10	above 10
Fecal urobilinogen, Ehrlich units	Watson *et al.*[41]	above 30	30–10	9–5	below 5
Sedimentation rate, mm. in hour	Wintrobe and Landsberg[12]	below 10	10–20	21–30	above 30

usually is produced by deficient secretion of bile into the duodenum and does not depend upon liver cell damage.

In the specimens submitted for biopsy, the degree of change in the liver cells was graded, without knowledge of the clinical diagnosis or the laboratory findings, from 0 to 3+ using the following criteria:

Grade 0 (Fig. 1). The appearance and staining of the liver cells was, in general, uniform. Small variations of the size of the nuclei are physiologic since usually the nuclei in the mid-zonal region are larger than in other parts of the lobule. However, the size of the nuclei within the same zone was identical.

Grade 1 (Fig. 2). Moderate variations in the size of the cells and the size of the nuclei even in the same zone were noted. The cytoplasm was rather uniform and did or did not show the fine regular vacuolization and granulation caused by the presence of glycogen.

Grade 2 (Fig. 3). In addition to moderate irregularity of the arrangement of the cells in the liver cell cords with variations in the size of the cells or the nuclei, at least one of the following two conditions was present: (a) nuclear alterations of moderate degree, such as pyknosis, karyolysis or ballooning, (b) cytoplasmatic changes of moderate degree, such as coarse meshes of the cytoplasm with vacuolization (vacuolated or plant cells of Gillman and Gillman[8]) or strongly basophilic or, more significant, eosinophilic areas in the cytoplasm producing a smudgy appearance or clumping of the cytoplasm due to coagulation necrosis (these clumps sometimes assume a branched character similar to the hyaline bodies described by Mallory[21]).

Grade 3 (Fig. 4). Marked degree of the changes listed under Grade 2, especially, (a) marked variations of the size of nuclei and cells, (b) high degree of the nuclear abnormalities described under grade 2, (c) marked acidophilic clumping with presence of many Mallory bodies or numerous liver cells revealing wide and empty meshes in the cytoplasm, and (d) anuclear cell fragments (ghost cells) or complete necrosis of cells.

If the liver cell alterations were not uniform, especially as in cirrhosis, the attempt was made to take an average of the entire picture. Focal necroses were not taken into account. Although the grading of morphologic characteristics is subjective, rereading of the same slides without knowledge of the grades previously given almost always showed identical results. In summary, in selecting grades 2 or 3, main emphasis was laid upon inequality and irregularity of the nuclei and vacuolization, but most especially upon acidophilia of the cytoplasm.

For statistical evaluation, the t values[6] were calculated to test the significance of the difference between the mean values of the results of hepatic tests in groups of patients with a given degree of morphologic liver cell damage. The t value was determined according to the following formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s} \sqrt{\frac{(n_1 + 1)(n_2 + 1)}{(n_1 + n_2 + 2)}}$$

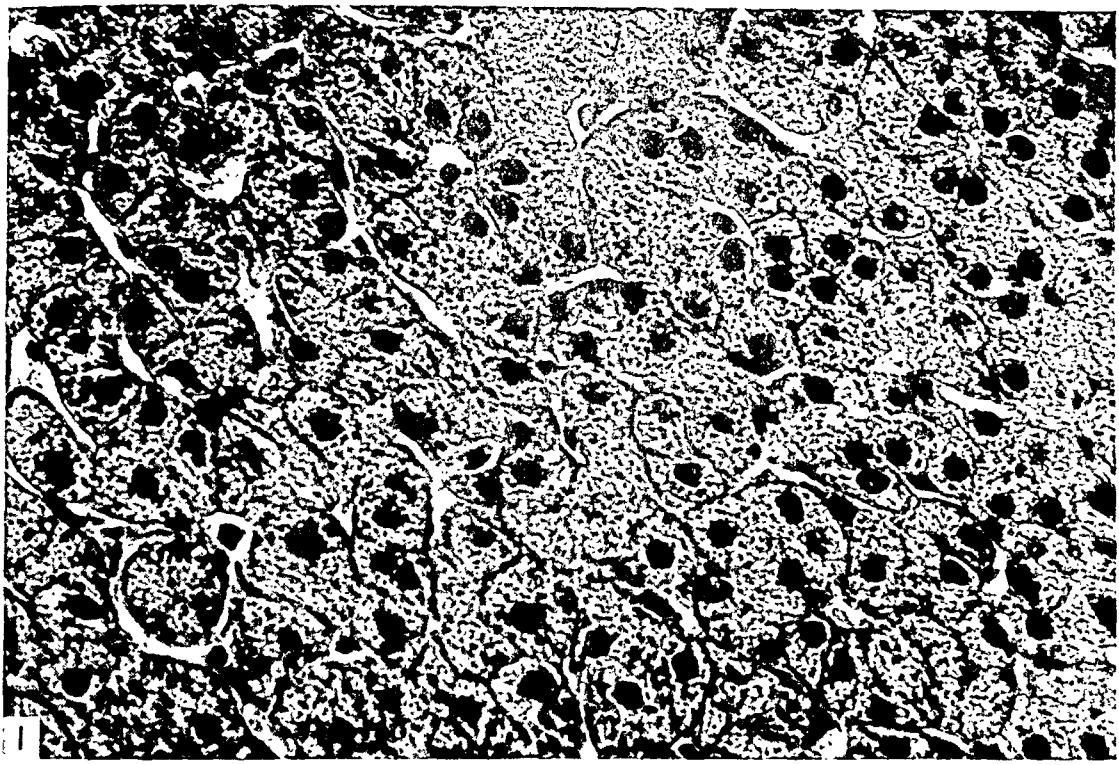
where $\bar{x}$ is the mean of determined values

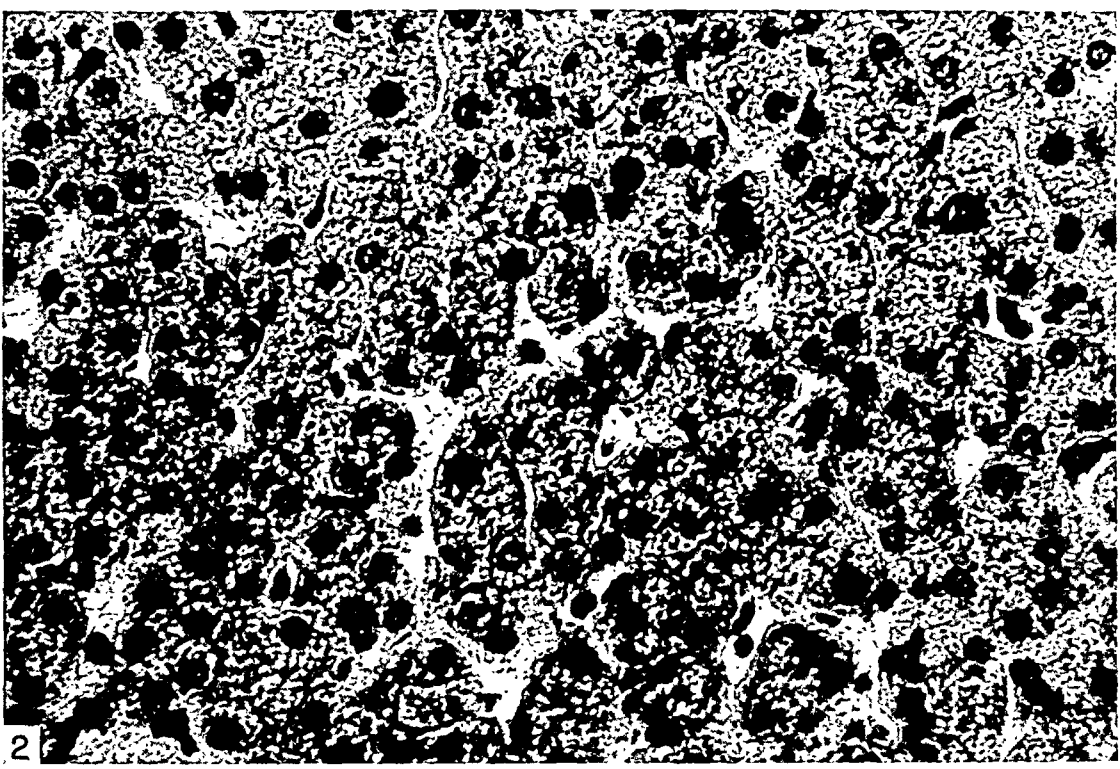
s is the standard deviation of determined value

n is the number of cases examined

FIG. 1. Photomicrograph of liver showing grade 0 liver cell damage. The liver cells and their nuclei are of about equal size. They are regularly arranged and their cytoplasm is uniformly finely granulated and vacuolated due to the presence of glycogen. × 540.

FIG. 2. Photomicrograph of liver showing grade + liver cell damage. The liver cells and their nuclei vary in size and shape and their arrangement is less orderly than in Figure 1. The cytoplasm also appears less uniform. × 540.





FIGS. 1–2

A t value of over 2.0 indicates a level of 5 or more per cent of significance (1:20 chance that the difference is significant). A t value above 2.7 indicates a 1 per cent level of significance (1:100 chance).

In addition, the chi square[33] was used to measure the association between the changes in degree of morphologic liver cell damage with the changes in degree of abnormality in the hepatic tests in the three groups with 1+, 2+ and 3+ liver cell damage. The group with no liver cell damage was not taken into consideration because of the relatively small number of cases available. The chi square was determined according to the following formula:

$$x^2 = \Sigma\ (X\text{-}m)^2/m$$

where Σ is summation,

X is the number of frequencies in each class

m is the expected number

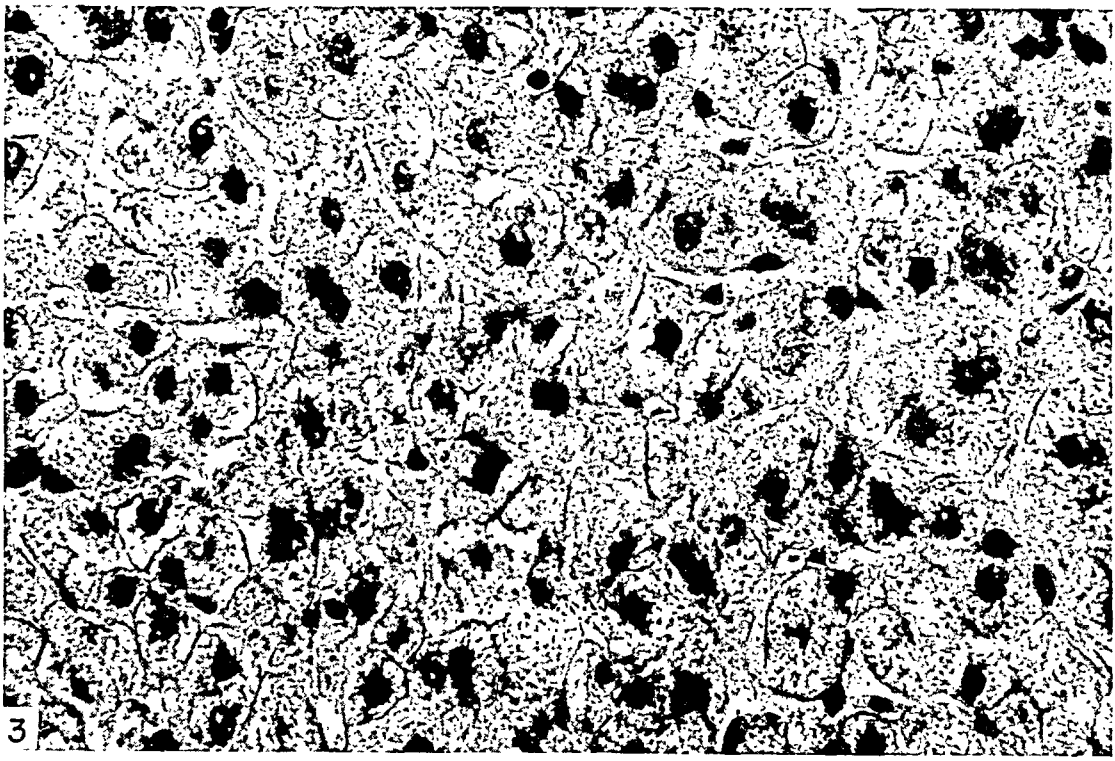
The expected frequencies were obtained by pooling the frequencies for the 3 groups. Therefore, a high value for chi square indicates a significant difference between the 3 groups of patients with different degrees of liver cell damage.

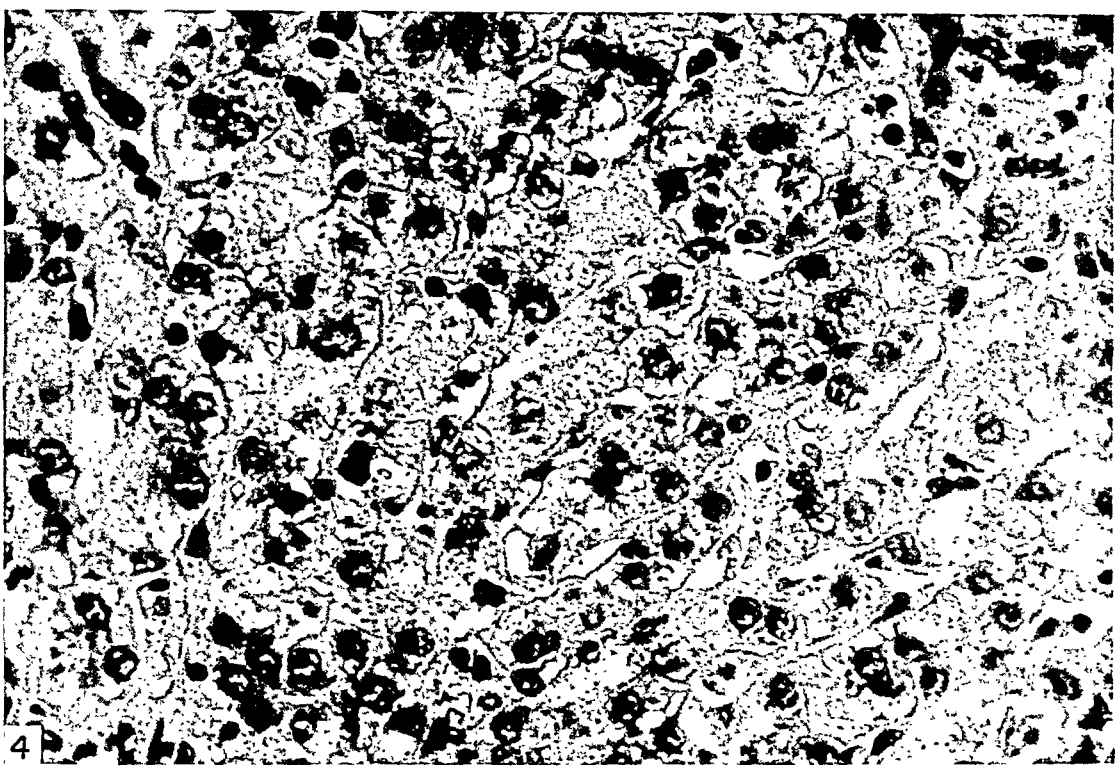
The association of the changes in degree of liver cell damage with degree of abnormality in the hepatic tests may be considered statistically significant with a chi square of 12.6 or over (5 per cent level of significance or 1:20 chance of significance of association) and even more significant with a chi square of 16.8 or over (1 per cent level of significance-1:100 chance of a significance of association). The difference in the character of the 3 groups is supported by significant t values.

The material was obtained from patients with (a) infectious and toxic hepatitis; (b) cirrhosis with and without jaundice (no attempt was made in the small biopsy specimens to distinguish the different anatomic types of cirrhosis); (c) extrahepatic biliary obstruction without infection (the liver cell damage almost invariably present was called biliary hepatitis); (d) extrahepatic biliary obstruction with secondary bacterial infection of the portal triads in, or more often around, the bile ducts (purulent hepatitis); and (e) various types of gastro-intestinal disorders in which a laparotomy was performed. The latter group provided the only few examples of absence of liver cell damage. Occasionally, one, and in some cases up to 4, of the total number of tests were not performed. In the further percentage evaluation, this small number of missing tests is taken into account.

FIG. 3. Photomicrograph of liver showing grade ++ liver cell damage. There is marked variation of the size and shape of the liver cells and their nuclei. The staining qualities of the nuclei vary. The cytoplasm of the liver cells appears in circumscribed foci smudgy and dark. In others, it is markedly ballooned due to hydropic degeneration. × 540.

FIG. 4. Photomicrograph of liver showing grade +++ liver cell damage. The arrangement of the liver cells is disorderly. The variations in size, shape and straining qualities of the liver cells and their nuclei are extremely marked. There are many areas of smudgy appearance of the cytoplasm caused by marked eosinophilia. There are also others of hydropic swelling. The nuclear staining is missing from some of the cells indicating necrosis. × 540.





FIGS. 3–4

RESULTS

Table 2 shows the mean of the results of the hepatic tests in each group of liver damage with statistical evaluation of their differences. In general, the more marked the degree of liver cell damage, the higher are the values of cephalin flocculation, thymol turbidity, serum globulin, alkaline phosphatase, urinary urobilinogen concentration, and total and one minute (direct) bilirubin. On the

TABLE 2

DEGREE OF MORPHOLOGIC LIVER CELL DAMAGE AND AVERAGE OF RESULTS OF HEPATIC TESTS, WITH STATISTICAL ANALYSIS OF SIGNIFICANCE OF CORRELATION

	MEAN OF RESULTS OF HEPATIC TESTS IN GROUPS WITH LIVER CELL DAMAGE GRADED AS				t TEST FOR DIFFERENCE OF GROUPS WITH		Chi Square
	0	+	++	+++	0 from + or more Liver Cell Damage	+ from ++ or more Liver Cell Damage	
Number of biopsy specimens studied	7	84	112	54			
Cephalin cholesterol flocculation	0.3+	0.6+	1.7+	2.3+	2.2	5.3	36.1
Thymol turbidity, units	5.5	5.4	8.2	10.8	0.1	4.1	26.2
Total protein, Gm./100 ml. serum	7.0	6.5	6.5	6.4	2.0	0.3	12.9
Albumin, Gm./100 ml. serum	4.6	3.7	3.3	3.0	2.0	3.6	21.2
Globulin, Gm./100 ml. serum	2.8	2.8	3.2	3.4	2.8	2.5	14.0
Albumin/globulin ratio	2.0	1.5	1.2	1.0	2.2	3.7	16.4
Nonprotein nitrogen, mg./100 ml. serum	29.6	33.1	34.2	33.5	1.5	1.5	5.6
Total cholesterol, mg./100 ml. serum	292	293	289	245	0.2	1.3	3.0
Total serum bilirubin, mg./100 ml. serum	0.4	6.9	12.8	13.5	6.1	3.0	20.9
1 minute (direct) serum bilirubin, mg./100 ml. serum	0.2	2.8	5.2	5.8	3.1	3.9	19.7
Prothrombin time, per cent	100,0	91.2	90.7	89.8	7.7	0.1	4.1
Serum alkaline phosphatase, Bodansky units	5.2	14.8	13.6	15.0	4.6	0.9	15.5
Urinary urobilinogen, Ehrlich units	0.3	3.0	6.5	4.1	3.7	1.2	7.0
Fecal urobilinogen, Ehrlich units	116.0	71.0	79.5	73.8	2.9	0.5	10.0
Sedimentation rate, mm./hour	18.5	29.4	30.0	30.5	1.5	0.1	2.0

basis of the chi square test, this association in this series is significant with results of cephalin flocculation, thymol turbidity and the bilirubin tests and just on the borderline of significance with results of the urinary urobilinogen test. An inspection of the individual groups reveals a significant difference between normal and slight liver cell damage for cephalin flocculation, globulin, alkaline phosphatase, urinary urobilinogen and bilirubin tests but not for the thymol turbidity test. With thymol turbidity, serum globulin and alkaline phosphatase

tests, the mean values were above the level considered normal even in the instances without liver damage while the results of cephalin flocculation and urinary urobilinogen concentration tests were still in the normal range even in the group with 1+ liver damage. The difference between the group with 1+ liver damage and those with 2 and 3+ was significant with cephalin flocculation, thymol turbidity, globulin and the bilirubin tests but not with urinary urobilinogen and especially not with the alkaline phosphatase test.

In general, the more marked the liver cell damage, the lower was the serum albumin and the albumin/globulin ratio, the fecal urobilinogen value and the prothrombin-time percentage. The chi square formula supports this relationship in the former 2 but not in the latter 2. The difference between no liver damage and mild liver damage is significant with the prothrombin-time percentage and also with results of the fecal urobilinogen test but not with values for albumin and albumin/globulin ratio. However, none of them were in the range of abnormality. The difference between mild and marked liver damage was significant in results of the former 2 tests but not in the latter 2 tests. The average in prothrombin-time percentage and fecal urobilinogen concentration was not in the abnormal range in any group.

There was no significant parallelism between liver cell damage and the results of the hepatic tests in total protein, total cholesterol, nonprotein nitrogen and sedimentation rate. The mean of the total cholesterol and sedimentation rate was in the abnormal range even in the group without liver cell damage.

A correlation of the number of hepatic tests yielding abnormal results with the degree of liver cell damage has been attempted in all instances in which at least 10 of the hepatic tests have been performed (Fig. 5). In general, the more marked the liver damage the higher was the number of hepatic tests giving abnormal results. In the majority of cases with 3+ liver cell damage, 8 or 9 of the tests were positive. However, in no group were all, or none, of the tests or quotients abnormal.

As a rule, the parallelism between the results of the hepatic tests and the morphologic liver cell damage was similar in the different pathologic conditions studied. Figure 6 shows the degrees of deviation in 3 tests in 6 different types of disease. The results of the cephalin flocculation and of the thymol turbidity tests, in general, paralleled the degree of liver cell damage in most of the hepatic conditions studied. However, in biliary hepatitis, this parallelism was lacking, since even with the severest degree of liver cell damage, the cephalin flocculation was not abnormal and the thymol turbidity only mildly elevated. Severe liver cell damage in extrahepatic biliary obstruction may, therefore, be associated with normal or nearly normal flocculation tests. If a bacterial infection complicated the extrahepatic biliary obstruction (purulent hepatitis), the flocculation tests became highly abnormal and parallelled the degree of liver cell damage. The elevation of the alkaline phosphatase in both groups of extrahepatic biliary obstruction was higher than in the nonsurgical diseases and did not parallel the degree of liver cell damage. This implies that biliary obstruction is a more important factor in producing marked elevation of the alkaline phosphatase than is liver cell damage.

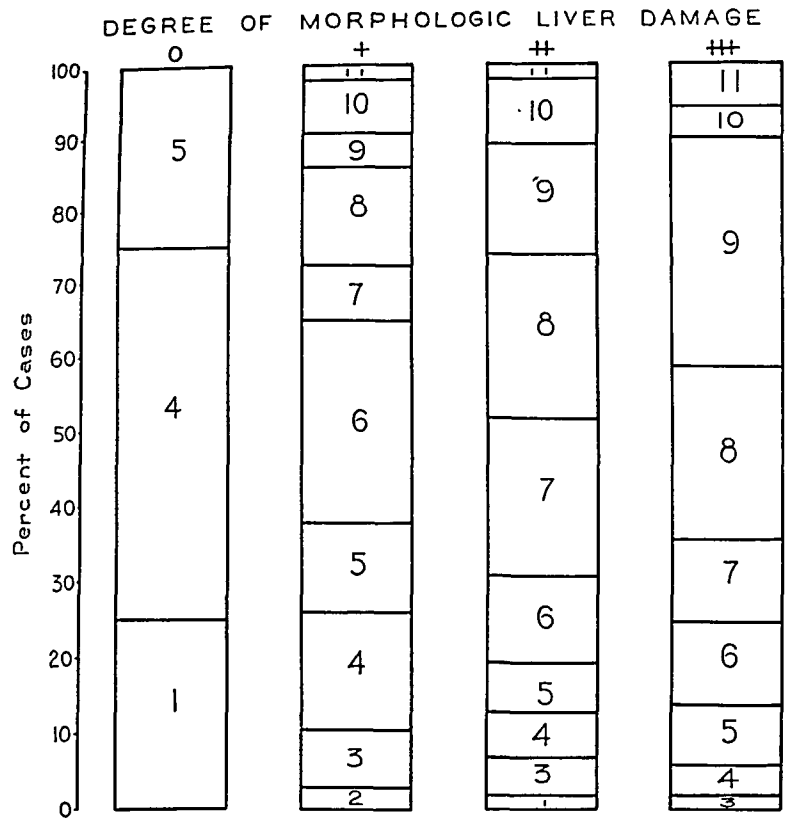


FIG. 5. Relation of degree of liver cell damage, indicated by 0 or plus signs, to the number and indicated in the columns of abnormal results in the different hepatic tests. The portion of the column marked by the numbers represents the percentage of cases having the specified number of abnormal results.

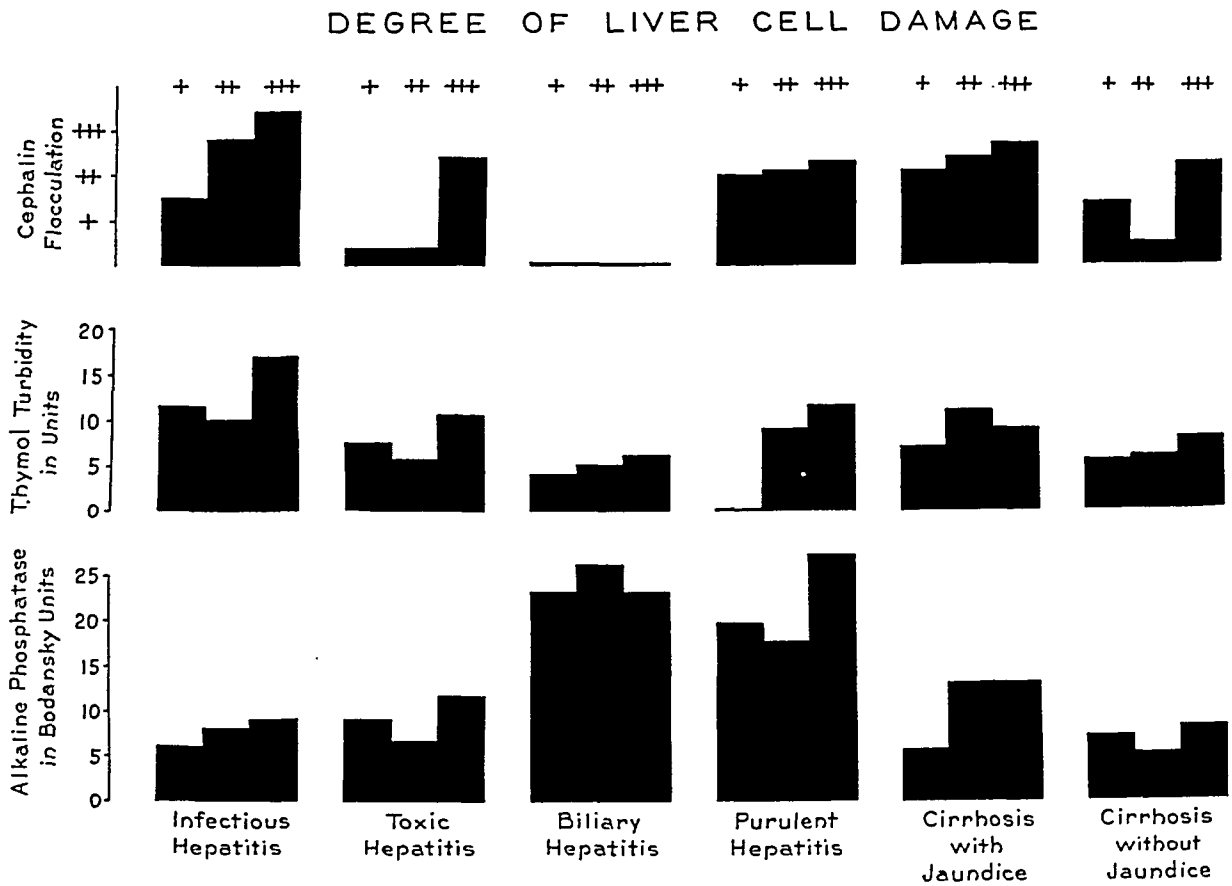


FIG. 6. Average of results of hepatic tests (as indicated by the height of the column) in groups of patients with various degrees of liver damage, in different hepatobiliary diseases.

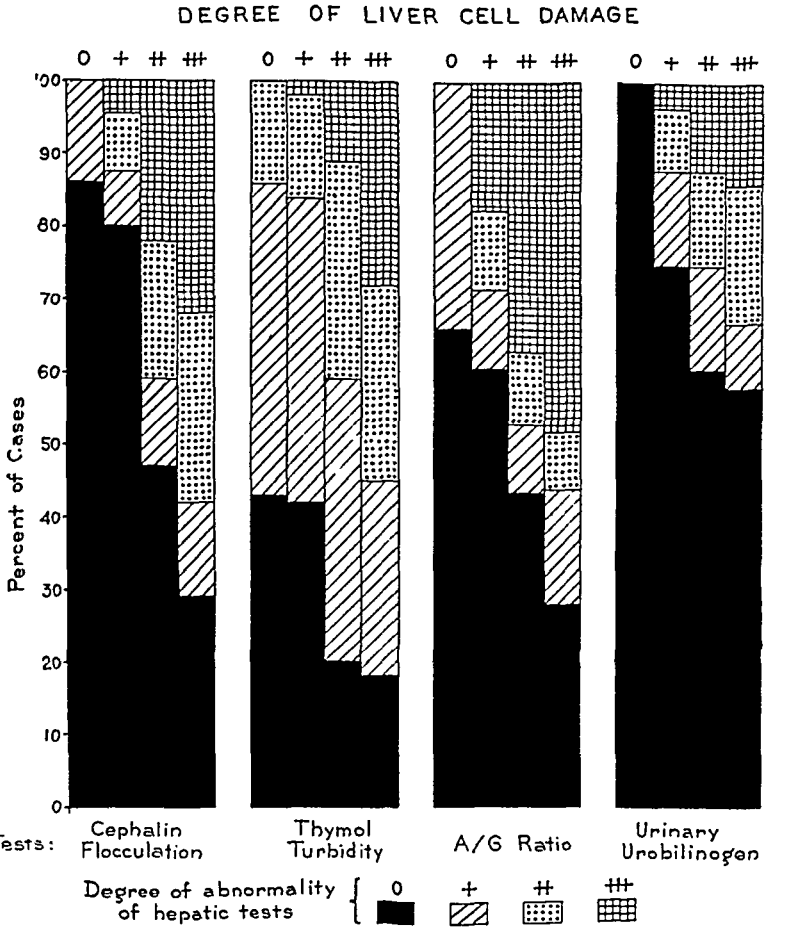


FIG. 7. Relation of degree of liver cell damage to degree of abnormality of hepatic tests expressed in per cent of cases.

TABLE 3

BREAKDOWN OF CASES STUDIED WITH VARYING DEGREE OF LIVER CELL DAMAGE ACCORDING TO RESULTS OF CEPHALIN CHOLESTEROL FLOCCULATION AND URINARY UROBILINOGEN CONCENTRATION

RESULTS OF TESTS	DEGREE OF LIVER CELL DAMAGE				
	0	+	++	+++	Total
Normal cephalin flocculation, urinary urobilinogen not elevated	6	51	41	11	22.0
Cephalin flocculation abnormal, urinary urobilinogen not elevated	1	8	25	21	42.0
Cephalin flocculation normal, urinary urobilinogen elevated	0	9	12	4	8.0
Cephalin flocculation abnormal, urinary urobilinogen elevated	0	5	27	14	28.0

The correlation between the results of the hepatic tests and the degree of liver cell damage was further analysed by recording in each morphologic group the degree of abnormality of cephalin flocculation, thymol turbidity, albumin globulin ratio and urinary urobilinogen. These tests were selected as the most informative. Besides serving as an additional illustration of the close association between the results of these tests and liver function damage, Figure 7 provides graphic illustration as to the value of the tests in screening for liver damage. It appears from this analysis that abnormal results in cephalin flocculation and increase in urinary urobilinogen suggest the presence of liver cell damage whereas normal results do not necessarily exclude it. This is primarily due to the normal cephalin flocculation in non-infected extrahepatic biliary obstruction and to the reduction of urinary urobilinogen in biliary obstruction, intra- and extrahepatic in nature, even in the presence of severe liver cell damage. In the presence of little or no liver cell damage, the results of both cephalin flocculation and urinary urobilinogen together (Table 3) were only rarely, if at all, abnormal whereas in the group of marked liver cell damage, both were normal in a considerable percentage of instances.

DISCUSSION

Damage to the liver cord cells is only one of the possible pathologic alterations of the liver but the one of special interest to the clinician. It is the type of damage which the liver function tests are supposed to mirror. However, some factors militate against this possibility. In contrast to the renal function tests, hardly any of the hepatic tests in common use (with the possible exception of the prothrombin response to vitamin K administration) are true function tests in the sense that they measure a basic function which is peculiar to the liver.[24] In addition, the great regenerative ability as well as functional reserve of the liver may prevent recognition of even severe morphologic liver cell damage.[14] Nevertheless, a statistical analysis reveals that the results of some of the hepatic tests mirror the degree of liver cell damage even if in individual cases this correlation does not necessarily hold true.

Although such a correlation indicates only an association and not necessarily that the liver cell damage causes the alteration of the hepatic tests, it is important from a practical diagnostic viewpoint to establish which hepatic tests correlate best with the degree of liver cell damage. According to the statistical analysis presented, the results of some tests (total serum protein, total serum cholesterol, fecal urobilinogen, nonprotein nitrogen, prothrombin-time percentage and sedimentation rate) had no significant association. Other tests, like urinary urobilinogen and alkaline phosphatase, show a correlation only concerning the absence or presence of liver cell damage but did not indicate the degree of damage. With the serum bilirubin, albumin, globulin and flocculation tests, the correlation is good in all grades of liver cell damage.

Urinary urobilinogen failed to reveal a difference between mild and marked liver cell damage because biliary obstruction reduces the urinary urobilinogen even in the presence of severe liver cell damage.[34, 38] The faulty take-up of uro-

bilinogen from the portal circulation by the damaged liver cells which results in increased urinary excretion of urobilinogen cannot make itself felt in the absence of bilirubin from the intestine.

Similarly, the alkaline phosphatase level is correlated only with absence or presence of liver cell damage and not with the degree of damage, when present. Biliary obstruction independent of liver cell damage raises the phosphatase level markedly (above 15 Bodansky units),[2, 9] whereas liver cell damage as such, independent of biliary obstruction, is associated with mild degree of phosphatase elevation (between 4 and 15 Bodansky units). The older concept that elevation of alkaline phosphatase in liver diseases is due to deficient biliary clearance of serum phosphatase is now replaced by the idea that the elevated phosphatase is formed by the liver. Its marked elevation in obstructive, and its moderate elevation in non-obstructive liver cell damage are now related to either increased formation in the liver or regurgitation of hepatogenic enzyme into the blood.[4, 5, 28]

The close association of the elevation of total and one minute (direct) serum bilirubin with liver cell damage was expected, though the accumulation of biliary substance in the blood in the obstructive form may be the cause of the liver cell damage while in the hepatogenous form liver cell damage may have caused the bilirubin elevation. However, this latter point is questionable since regurgitation of bile pigments is now considered to occur through the cholangioles rather than through the hepatic cells.[10] The ratio between total and direct bilirubin itself is not related to liver cell damage.[29]

The most significant correlation seems to be furnished by the results of the serum protein partition into albumin and globulin and the flocculation tests related to this partition. The association between liver cell damage and the serum albumin level is easily explained by the formation of albumin by the liver cells.[20] The serum globulin elevation which parallels the degree of liver cell damage cannot be explained simply by liver cell damage itself since the gamma-globulins which mainly cause the elevation are not formed by the liver cells. The elevation is better explained by increased globulin formation by mesenchymal cells, especially Kupffer cells which proliferate in many forms of liver cell damage. Since both cephalin flocculation and thymol turbidity depend upon reduction of alalbumin and elevation of gamma-globulin[15, 19] (lipoproteins and lipids playing an additional role in the thymol turbidity[16]), the good correlation between liver cell damage and the flocculation tests is to be expected. The cases without liver cell damage in this series suffered from gastrointestinal diseases which by themselves may elevate the thymol turbidity. This accounts for the slightly abnormal levels seen in almost all cases studied.

Previously[25] it had been concluded that the more severe the liver cell damage, the greater the number of hepatic tests with abnormal results, independent of the physiologic basis of the tests. This assumption has been confirmed in this study by comparison with the histologic appearance of the liver. At the same time, it was demonstrated that in no instance were all tests positive even in the presence of most severe liver cell damage. This indicates the advantage of doing several hepatic tests to establish the presence and degree of liver cell damage.

This is especially advisable since the performed hepatic tests have a significant incidence (about 10 per cent[24]) of false-positive or negative reactions (abnormal results in the absence or normal results in the presence of liver cell damage).

Study of the correlations in various diseases reveals significant exceptions. The most important is that in liver cell damage produced by non-infected extrahepatic biliary obstruction the cephalin flocculation test is normal and the thymol turbidity only slightly elevated even in the presence of severe damage. A possible explanation for this phenomenon is the absence of gamma-globulin elevation in this condition.[17] The latter is in turn explained by the absence of the increased gamma-globulin formation by proliferated mesenchymal, especially Kupffer, cells which is characteristic for almost all other liver diseases. Morphologic evidence seems to support this assumption since in contrast to almost all other liver diseases, the mesenchymal reaction is not prominent in non-infected extrahepatic biliary obstruction and, where present, is associated with severe bile imbibition of the Kupffer cells. Cytoplasmatic ribonucleic acid, indicating protein formation, has been demonstrated in the mesenchymal cells in most primary types of hepatitis and cirrhosis as well as in purulent hepatitis but not in non-infected extrahepatic biliary obstruction.[35]

The great diagnostic value of the flocculation tests lies in the ability of the latter to differentiate liver cell damage in extrahepatic biliary obstruction from other forms of liver cell damage. The most significant exception is secondary bacterial infection of the portal triads, *e.g.*, as in cholelithiasis (purulent hepatitis). In this condition, parallel with a marked mesenchymal reaction, the results of the flocculation tests are or become abnormal.[23, 26] However, this event can usually be recognized by clinical evidence of septicemia (i.e., by fever, chills, and leukocytosis) and therefore should not cause diagnostic difficulties.

The analysis presented indicates that of the performed tests, a combination of the cephalin flocculation test with the determination of urinary urobilinogen appears to be most helpful in screening cases that probably have morphologically recognizable liver cell damage. Positive results strongly suggest its presence; negative ones, however, do not exclude it (if produced by extrahepatic biliary obstruction).

From a general pathologic point of view, our experiences here discussed lead to a more optimistic view, than is usually held regarding the ability to recognize in biopsies morphologic changes which reflect functional damage of the liver cells and *vice versa*, regarding the possibility to predict morphologic changes from the results of the hepatic tests.

SUMMARY

The degree of morphologically recognizable liver cell damage as seen in biopsies of liver tissue was statistically correlated with the degree of abnormality in results of a series of hepatic tests performed at the time the liver tissue was removed for biopsy. The degree of liver cell damage revealed a statistically significant correlation with an increase in cephalin flocculation, thymol turbidity, total and direct bilirubin and with a decrease in albumin, and albumin/globulin ratio. Elevation of values of alkaline phosphatase and urinary urobilinogen tests

was associated with the presence of liver cell damage but not with its degree. Analysis of the results of total serum protein, nonprotein nitrogen, fecal urobilinogen, prothrombin-time percentage, sedimentation rate and total serum cholesterol tests yielded no significant correlation.

The relation of liver cell damage to alkaline phosphatase and urobilinogen tests is obscured by the fact that the value of the former is elevated and the latter reduced by biliary obstruction independent of liver cell damage. The association with decrease in albumin is explained by its formation in the liver cells. The correlation with the increase in globulin, however, is considered due to the mesenchymal reaction (chiefly Kupffer cells) associated with many forms of hepatocellular injury, the chief exception being extrahepatic biliary obstruction without infection. This accounts not only for the usually close correlation between the results of the flocculation tests and the degree of liver cell damage but also for the normal results in the flocculation tests in noninfected extrahepatic biliary obstruction, even in the presence of severe liver cell damage. This fact makes the flocculation tests especially valuable in the differential diagnosis between surgical and medical types of jaundice. However, they become positive if secondary bacterial infection complicates extrahepatic biliary obstruction.

The number of hepatic tests with abnormal results independent of their physiologic basis rises with the degree of liver cell damage. For the purpose of screening for the presence of liver cell damage a combination of the cephalin flocculation and urinary urobilinogen tests is of value in that positive results indicate liver cell damage while negative results do not exclude it in the presence of biliary obstruction.

Acknowledgment. The statistical analyses were performed by Miss Elizabeth M. Adles. Most of the needle aspirations were made by Dr. D. D. Kozoll and most of the surgical specimens were obtained by Dr. K. A. Meyer.

REFERENCES

1. Bloor, W. R., and Knudson, A.: The separate determination of cholesterol and cholesterol esters in a small amount of blood. J. Biol. Chem., **27:** 107–112, 1916.
2. Bodansky, A.: Phosphatase studies: determination of serum phosphatase: factors influencing accuracy of determination. J. Biol. Chem., **101:** 93–104, 1933.
3. Buck, R. E.: Observations on alcoholic fatty liver; the use of interval needle biopsy and liver function tests. J. Lab. and Clin. Med., **33:** 555–564, 1948.
4. Cantorow, A., and Miller, L. L.: Non-excretion of jaundice—serum alkaline phosphatase in bile of normal dogs. Am. J. Physiol., **153:** 444–446, 1948.
5. Freeman, S., and Chen, Y. P.: The effect of jaundiced blood upon normal dogs with special reference to the serum phosphatase. J. Biol. Chem., **123:** 239–246, 1938.
6. Fisher, R. A.: Statistical Method for Research Workers. Ed. 10. London: Oliver & Boyd, Ltd., 1948.
7. Franklin, M., Popper, H., Steigmann, F., and Kozoll, D. D.: Relation between structural and functional alterations of the liver. J. Lab. and Clin. Med., **33:** 435–447, 1948.
8. Gillman, J., and Gillman, T.: Anoxia and the liver with special reference to shock and chronic malnutrition. South African J. M. Sc., **13:** 11–43, 1948.
9. Gutman, A. B., Olson, K. B., Gutman, E. B., and Flood, C. A.: Effect of disease of the liver and biliary tract upon the phosphatase activity of the serum. J. Clin. Investigation, **19:** 129–152, 1940.
10. Hanger, F. M.: Serologic differentiation of obstructive from hepatogenous jaundice by flocculation of cephalin cholesterol emulsion. J. Clin. Investigation, **18:** 261–269, 1939.
11. Hoffbauer, F. W., Evans, G. T., and Watson, C. J.: Cirrhosis of the liver with particular reference to correlation of composite liver function studies with liver biopsy. M. Clin. North America, **29:** 363–388, 1945.
12. Hoffman, W. S.: Photelometric Clinical Chemistry. New York: William Morrow & Company, 1944.

13. Howe, P. E.: The determination of proteins in blood—a micro method. J. Biol. Chem., **49:** 109–113, 1921.
14. Ivy, A. C., and Roth, J. A.: Why do a liver function test? Gastroenterology, **1:** 655–668, 1943.
15. Kabat, E. A., Hanger, F. M., Moore, D. H., and Landow, H.: The relation of cephalin flocculation and colloidal gold reactions to the serum proteins. J. Clin. Investigation, **22:** 563–568, 1943.
16. Kunkel, H. G., and Hoagland, C. L.: Mechanism and significance of the thymol turbidity test for liver disease. J. Clin. Investigation, **26:** 1060–1071, 1947.
17. Luetscher, J. A., Jr.: Biological and medical applications of electrophoresis. Physiol. Rev., **27:** 621–642, 1947.
18. Maclagan, N. F.: Thymol turbidity test; a new indicator of liver dysfunction. Nature, London: **154:** 670–671, 1944.
19. Maclagan, N. F., and Bunn, D.: Flocculation tests with electrophoretically separated serum proteins. Biochem. J., **41:** 580–586, 1947.
20. Madden, S. C., and Whipple, G. H.: Plasma proteins: their source, production and utilization. Physiol. Rev., **20:** 194–217, 1940
21. Mallory, T. B.: Cirrhosis of the liver; five different types of lesions from which it may arise. Bull. Johns Hopkins Hosp., **22:** 69–75, 1911.
22. Mateer, J. G., Hartman, F. W., Baltz, J. I., Fallis, L. D., McGraw, A. B., and Steele, H. H.: Combined liver biopsy and liver function study in 132 cases of cholelithiasis and 31 cases of peptic ulcer. Gastroenterology, **11:** 284–302, 1948.
23. Popper, H., and Franklin, M.: Differential diagnosis of hepatitis by histologic and functional laboratory methods. J. A. M. A., **137:** 230–234, 1948.
24. Popper, H., and Schaffner, F.: Hepatic Tests. Advances in Internal Medicine. Vol. 4. New York: Interscience Publishers, Inc., in press.
25. Popper, H., and Steigmann, F.: Differential diagnosis between medical and surgical jaundice by laboratory tests. Ann. Int. Med., **29:** 469–487, 1948.
26. Popper, H., Steigmann, F., Meyer, K. A., Kozoll, D. D., and Franklin, M.: Correlation of liver function and liver structure; clinical applications. Am. J. Med., **6:** 278–291, 1949.
27. Quick, A. J.: Determination of prothrombin. Proc. Soc. Exper. Biol. and Med., **42:** 788–789, 1939.
28. Rapoport, S.: Increased serum phosphatase and "hyperprothrombinemia" in infectious hepatitis of children. Proc. Soc. Exper. Biol. and Med., **62:** 203–207, 1946.
29. Schaffner, F., Popper, H., and Steigmann, F.: Prompt and indirect reacting serum bilirubin in jaundice. Federation Proc., **8:** 367, 1949.
30. Shank, R. E., and Hoagland, C. L.: A modified method for the quantitative determination of the thymol turbidity reaction of the serum. J. Biol. Chem., **162:** 133–138, 1946.
31. Sherlock, S. P. V.: Biochemical investigations in liver disease; some correlations with hepatic histology. J. Path. and Bact., **58:** 523–544, 1946.
32. Sherlock, S., and Walshe, V.: Effect of undernutrition in man on hepatic structure and function. Nature, **161:** 604, 1948.
33. Snedecor, G. W.: Statistical Methods. Ames, Iowa: Collegiate Press, Inc., 1937.
34. Steigmann, F., and Dyniewicz, J. M.: Studies of urobilinogen; quantitative urobilinogen determination in the differential diagnosis of jaundice. Gastroenterology *1*, 855–875, 1943.
35. Szanto, P. B., and Popper, H.: Ribonucleic acid distribution in hepatic diseases. In preparation.
36. Turkel, H., and Bethell, F. H.: A new and simple instrument for administration of fluids through bone marrow. War Med., **5:** 222–225, 1944.
37. Volwiler, W., Jones, C. K., and Mallory, T. B.: Criteria for the measurements of results of treatment in fatty cirrhosis. Gastroenterology, **11:** 164–182, 1948.
38. Watson, C. J.: Studies of urobilinogen; per diem excretion of urobilinogen in common forms of jaundice and disease of liver. Arch. Int. Med., **59:** 206–231, 1937.
39. Watson, C. J., and Ducci, H.: The quantitative determination of the serum bilirubin with special reference to the prompt reacting and the chloroform soluble types. J Lab. and Clin Med., **30:** 293–300, 1945.
40. Watson, C. J., and Hoffbauer, F. W.: The problem of prolonged hepatitis with particular reference to the cholangiolitic type and to the development of cholangiolitic cirrhosis of the liver. Ann. Int. Med., **25:** 195–227, 1946.
41. Watson, C. J., Schwartz, S., Sborov, V., and Bertie, E.: A simple method for the quantitative recording of the Ehrlich reaction as carried out with urine and feces. Am. J. Clin. Path., **14:** 605–615, 1944.
42. Wintrobe, M. M., and Landsberg, J. W.: A standardized technique for the blood sedimentation test. Am. J. M. Sc., **189:** 102–115, 1935.

SARCOIDOSIS

A Clinicopathologic Review of Three Hundred Cases, Including Twenty-Two Autopsies*

WALTER RICKER, M.D., and MARDELLE CLARK, A.B.

From the Army Institute of Pathology, Washington, D. C., and the Department of Pathology of the University of Washington School of Medicine, Seattle, Washington

From the files of the Army Institute of Pathology three hundred consecutive cases with lesions diagnosed as sarcoidosis were analysed and reviewed in the preparation of this report. Criteria for the diagnosis early in the study corresponded to the classical concept of the disease, best expressed as a "Definition of Sarcoidosis" by the Conference on Sarcoid, National Research Council, February 11, 1948, as follows:

"Sarcoidosis is a disease of unknown etiology. Pathologically it is characterized by the presence in any organ or tissue of epithelioid cell tubercles with inconspicuous or no necrosis and by the frequent presence of refractile or apparently calcified bodies in the giant cells of the tubercles. The characteristic lesions may be replaced by fibrosis, hyalinization or both.

"Clinically, the lesions may be widely disseminated. The tissues most frequently involved are lymph nodes, lungs, skin, eyes and bones, particularly of the extremities.

"The clinical course usually is chronic with minimal or no constitutional symptoms; however, there may be acute phases characterized by a general reaction with malaise and fever. There may be signs and symptoms referable to the tissues and organs involved.

"The intracutaneous tuberculin test is frequently negative; the plasma globulins are often increased.

"The outcome may be clinical recovery with radiographic evidence of residue, or impairment of function of organs involved, or a continued chronic course of the disease."

Reference will be made only to those publications which pertain to this analysis. Those who wish to consult more extensive reviews of the literature are referred to the articles of Pinner,[13] Harrell,[7] Longcope,[11] Katz, Cake and Reed,[10] Rubin and Pinner,[17] Reisner,[15] and Freiman.[4]

HISTOLOGIC FEATURES

As this study progressed, there emerged a histologic pattern which was adopted as the criteria for the pathologic diagnosis. Most of this material has been selected on the basis of these histologic features. Discrete epithelioid granulomas make up the lesions. Usually these occur in lympho-reticular tissue, but lung, liver, skin, bone and other organs are not infrequently involved. The characteristic granulomas tend to occur in clusters or "crops" of tubercles of the same stage of development, resulting in a pattern best described as monotonous. At all stages of development these granulomas preserve individual outlines, and

* Presented at the Twenty-Sixth Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 28, 1948.

Received for publication, May 31, 1949.

only in the final healing stages of fibrosis and hyalinization do they merge and become blurred.

The youngest granuloma is sharply delimited from the surrounding tissue and is composed of uniform, polyhedral cells with abundant, acidophilic cytoplasm and large, ovoid, vesicular nuclei (Fig. 4B). As the lesion matures, a narrow rim of 2 or 3 layers of fibroblasts encircle it while the epithelioid cells elongate and become concentrically arranged (Fig. 6). Regression by fibrosis then occurs, beginning as a fibrotic process between and around the individual granulomas (Fig. 5A). The fibrous tissue rapidly becomes compact, hyalinizes and forms a dense scar in which only a suggestion of the previous tubercle remains (Fig. 5B).

MATERIALS AND METHODS

During the screening of this material, lesions that were not characteristic were excluded from review. Such atypical lesions included granulomas of tuberculosis, syphilis and the mycoses, foreign body granulomas, lipoidal and lipophagic granulomas, and others of undertermined etiology. As will be seen from the review of case histories in this paper, 5 patients had associated tuberculosis with histologic criteria for that disease, and in the lesions of 3 of these patients, tubercle bacilli were demonstrated. Granulomas characteristic of sarcoidosis also occurred in these latter patients and, consequently, these examples were included in this review. It is possible that too fine a differentiation is being attempted although, in this material, histologic differences between miliary tuberculosis and the granuloma under discussion seem apparent.

Histologic sections from each case were stained with hematoxylin and eosin and with the Ziehl-Neelsen stain for acid-fast organisms. Other stains were made in smaller numbers, including phosphotungstic acid-hematoxylin, fluorescent stain for acid-fast organisms,[6] Giemsa's stain, the Brown and Brenn modification of Gram's stain and others. In 36 of the cases, cultures for tubercle bacilli were attempted and in 26 cases material was injected into guinea pigs in an effort to recover acid-fast organisms.

The acceptable cases were then analyzed from the clinical point of view, in part from examination of the clinical records of the patients comprising the study, and in part from follow-up questionnaires. The information thus obtained is grouped as follows: (1) data from surgical cases; (2) data from autopsied cases; (3) data obtained from a follow-up questionnaire sent to patients in the surgical group.

Surgical cases. Of the 300 patients, a total of 278 had been admitted to hospitals because of sarcoidosis or for other reasons, and had been discharged as living patients up to the time this study was begun. In this group, a definite diagnosis of sarcoidosis was made either from tissue removed for biopsy or from tissue removed during operation for some other cause.

Autopsied cases. The presence of sarcoidosis was either detected or confirmed at autopsy in 22 cases. Twelve of the patients who died were white and 10 were Negro; 21 were males, with ages ranging from 18 through 54 years. The clinical data from subjects on which autopsy was performed are included in the resumé of

clinical features of the entire group of 300 cases, while the pathologic data from these 22 cases are discussed in a separate section.

Information from questionnaires. Questionnaires sent to 114 patients in the surgical group contained the following inquiries: What was the patient's physical condition prior to hospitalization at the time sarcoidosis was diagnosed? Did he know of any similar illness of other persons in his vicinity at the time of his admission to the hospital? Had he had a recurrence of the same trouble after discharge? What was his opinion as to the cause of his illness? Of the 114 questionnaires, 67 replies were received by the time this report was written.

CLINICAL OBSERVATIONS

Certain limitations are inherent in the source from which most of the material was derived and must be taken into consideration in interpreting the data. For example, the fact that cases submitted to the Institute come chiefly from Army hospitals, weights the figures in terms of both age and sex, 271 of the 300 patients being among men of military age (from 18 to 39 years). However, the assembling of 271 cases from among the military personnel indicates a wider prevalence of sarcoidosis, especially among males, than has heretofore been demonstrated. A weakness in the data lies in the lack of uniformity and completeness in reports of clinical observations in the case histories. This is especially evident with respect to results of laboratory examinations; no information, or partial information only, was presented in a large number of the case records.

Age, Race, and Sex

Age. The age distribution of the 300 patients with sarcoidosis is shown in Table 1, and that of the white and Negro patients is also presented in graphic form in Figure 1. As would be expected in view of the preponderance of younger men in the Army, most of the patients were under 26 years of age. The median age of the entire group was 24.9 years; that of the white patients, 25.3 years, and of the Negroes, 24.4.*

When the percentage of patients in various age groups from 20 to 39 years of age is correlated with Army strength in these age groups, it is seen that the disease occurred nearly twice as often in soldiers between 20 and 24 years of age as in those between 30 and 40 years of age (Table 2).

The range in age among the entire group of 300 was greater for white persons than for Negroes. The youngest white patient was 7 years of age, the oldest 62; the youngest Negro was 17, the oldest 41.

Race. One hundred seventy-four of the 300 patients with sarcoidosis were Negroes; 113 were white; the color of 13 patients was not given. During the period covered by this study Negroes constituted 10 per cent of both the male strength of the Army and of the total number of men of military age in the

* The median age is used in order to minimize the effect of the extreme older ages in the entire group of patients; most of these older patients were not soldiers and, therefore, not fully representative of the group.

United States. Of the 271 Army patients in our study, 99 (or 36.5 per cent) were white and 172 (or 63.5 per cent) were Negro. By applying this figure to the estimated average annual Army strength during the period from January 1943 to June 1946, an incidence of 0.5 whites and 8.7 Negroes per 100,000 per year is obtained. Thus, the data indicate that the disease occurred 17 times as often among Negro men as among white men in the Army. Previous reports on the disease showed a higher proportion of Negroes than of white subjects among the cases studied, but not this overwhelming preponderance.[2, 5, 6]

Sex. Only 15 (or 5 per cent) of the patients whose sex was stated were women. This rate of occurrence in the two sexes is not significant because of the selectivity of the cases received at the Army Institute of Pathology.

TABLE 1

AGE DISTRIBUTION OF 300 PATIENTS WITH SARCOIDOSIS, BY RACE

AGE GROUP	NUMBER AND PER CENT OF PATIENTS		
	Total	White	Negro
7–19	15 (5.5)	3 (2.8)	12 (7.3)
20–24	125 (45.5)	45 (42.5)	77 (47.0)
25–29	79 (28.7)	29 (27.4)	49 (29.9)
30–34	32 (11.6)	17 (16.0)	14 (8.5)
35–39	13 (4.7)	5 (4.7)	8 (4.9)
40 and over	11 (4.0)	7 (6.6)	4 (2.4)
Total whose age and race			
Were known	275 (100.0)	106 (100.0)	164 (100.0)
Were unknown	25*	7	10

* In 8 cases, both age and race were unknown.

Nativity

Seventy-nine of 101 patients (78.2 per cent) were from the southeastern states. The preponderance of patients from these states can in part be explained by the high incidence in Negroes. However, since only 63.5 per cent of the entire series were Negroes, it would seem that the occurrence of the lesions preponderantly in persons born in the southern states may be of significance.

Symptomatology

Symptoms and signs. The signs and symptoms which were referable to sarcoidosis are presented in Table 3. No symptoms were reported for 42 (or 14 per cent) of the patients, the diagnosis having been made incidental to routine physical examination or at autopsy. The proportion of Negro patients (93 per cent) who reported symptoms was higher than that of white patients (76 per cent). Negroes had relatively more signs or symtoms than did the white patients; but the only signs and symptoms which appeared to be appreciably more frequent were weight loss and low grade fever. The percentage of each of these findings was nearly twice as high among Negro as among white patients.

Iridocyclitis also occurred in a much higher percentage of Negro than of white patients, but the number was too small for reliable statistical evaluation. It was the only sign in 1 of the 16 patients, and was accompanied by enlarged lymph nodes alone in 5. In the remaining 10 cases, there were associated general symptoms, such as fever, malaise, weight loss and weakness.

Duration of symptoms. Although patients' reports on the duration of symptoms are probably inadequate because of the lack of early recognition of such symp-

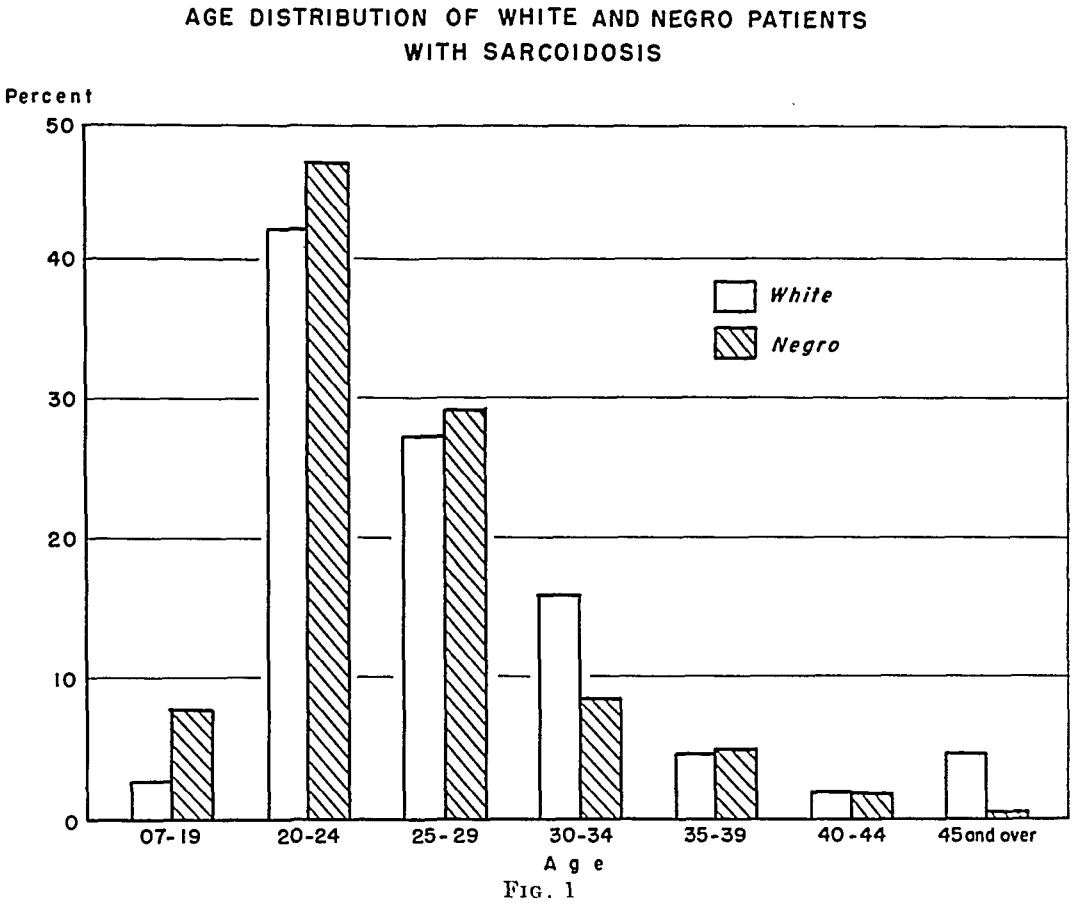


FIG. 1

toms, the known duration from onset to admission to the hospital averaged about 10 months. In 5 instances, death was sudden and without record of previous symptoms; in one case the known duration was 10 years. Nearly 70 per cent of the patients for whom the duration was known were admitted to hospitals within six months after the onset of symptoms.

The reported duration of symptoms in 184 cases by age is shown in Table 4. It is apparent that relatively more Negroes than white persons under 25 years of age were hospitalized within a period of less than five months after the onset of symptoms. The wide disparity between the races within equal age groups indicates that the age factor alone is not sufficient to account for early reporting.

It suggests the possibility either that in Negroes the symptoms became discernible or disturbing to a degree which led them to report for hospitalization at an earlier stage than the white patients, or that Negroes in the Army were more inclined to report symptoms of illness than were white soldiers.

TABLE 2

AGE DISTRIBUTION OF SARCOIDOSIS IN MEN 20–39 YEARS OF AGE IN RELATION TO ARMY STRENGTH

AGE	PATIENTS WITH SARCOIDOSIS		TOTAL ARMY STRENGTH*	RATE PER 100,000
	Number	Per Cent		
20–24	125	50.2	8142	1.5
26–29	79	31.7	7243	1.1
30–34	32	12.9	3811	0.8
35–39	13	5.2	2083	0.6

* Expressed in terms of man-hours of service.

TABLE 3

DISTRIBUTION OF SIGNS AND SYMPTOMS AMONG 258 PATIENTS WITH SARCOIDOSIS

SYMPTOMS	NUMBER AND PER CENT OF PATIENTS		
	Total*	White	Negro
Enlarged lymph nodes	221 (85.7)	78 (90.7)	132 (82.0)
Cough	51 (19.8)	15 (17.4)	35 (21.7)
Weight loss	48 (18.6)	11 (12.8)	36 (22.4)
Skin manifestations	40 (15.5)	17 (19.8)	22 (13.7)
Low grade fever	38 (14.7)	8 (9.3)	30 (18.6)
Chest pain	36 (14.0)	8 (9.3)	27 (16.8)
Malaise	26 (10.1)	7 (8.1)	18 (11.2)
Weakness	25 (9.7)	7 (8.1)	18 (11.2)
Symptoms of iridocyclitis	16 (6.2)	2 (2.3)	14 (8.7)
Swelling of bones and joints of extremities	7 (2.7)	3 (3.5)	4 (2.5)
Parotid swelling	5 (1.9)	2 (2.3)	3 (1.9)
Hemoptysis	3 (1.2)	1 (1.2)	2 (1.2)
Total patients with symptoms	258 (86.0)	86 (76.1)	161 (92.5)

* Includes 11 patients whose race was not stated.

Roentgenologic and Laboratory Observations

Even though the results of no single laboratory test or roentgenologic examination were recorded in the charts of all the 300 patients in the series, the number of each of a number of examinations made enough to provide data which contribute to an over-all concept of the disease. These data are presented and discussed in the following paragraphs.

Roentgenologic examinations. Roentgenograms of the chest were reported in 153 of the patients. Involvement of the mediastinum was noted in 116, or 76 per cent. There was a slightly higher incidence of mediastinal involvement recorded for Negroes (78 per cent) than for white subjects (71 per cent). Involvement of the lung parenchyma was found in 18 instances or in 12 per cent

TABLE 4

FREQUENCY OF SYMPTOMS IN 184 PATIENTS WITH SARCOIDOSIS, ACCORDING TO DURATION, AGE AND RACE

AGE GROUP	PERCENTAGE OF PATIENTS HAVING SYMPTOMS ACCORDING TO DURATION AND RACE			
	Less than 5 Months		5 Months and Over	
	White	Negro	White	Negro
0–24	42.0	61.5	46.9	49.1
25–29	29.0	24.6	25.0	30.2
30 and over	29.0	13.8	28.1	20.7

TABLE 5

ORGANS (EXCEPT LYMPH NODES) AFFECTED IN 195 CASES OF SARCOIDOSIS

ORGAN	NUMBER AND PER CENT OF PATIENTS AFFECTED		
	Total*	White	Negro
Lungs	70 (35.9)	24 (32.0)	45 (39.5)
Skin	41 (21.0)	17 (22.7)	21 (18.4)
Bone	34 (17.4)	12 (16.0)	19 (16.7)
Eye	17 (8.7)	1 (1.3)	16 (14.0)
Liver	17 (8.7)	9 (12.0)	8 (7.0)
Spleen	16 (8.2)	9 (12.0)	7 (6.1)
Kidneys	5 (2.6)	2 (2.7)	3 (2.6)
Brain and meninges	3 (1.5)	0	3 (2.6)
Heart	2 (1.0)	2 (2.7)	0
Skeletal muscles	2 (1.0)	1 (1.3)	1 (0.9)
Genito-urinary	1 (0.5)	0	1 (0.9)
Endocrine	1 (0.5)	0	1 (0.9)
Total with known organ involvement (except lymph nodes)	195	75	114

* Includes 6 patients whose race was not stated.

of the 153 so studied. Reports of roentgenograms of the small bones for 94 patients included the diagnosis of involvement, consistent with sarcoid, in 34 (36 per cent).

Recovery of acid-fast bacilli. In one autopsied case, acid-fast bacilli were recovered on guinea pig inoculation with lymph node emulsion after the patient had died. In 36 cases cultures for *Mycobacterium tuberculosis* were attempted and

in 26 cases material was injected into guinea pigs in an effort to recover acid-fast organisms. One positive result was obtained by each method.*

Tuberculin skin tests. Skin tests were made on 88 patients, with positive reactions in only 3 (or about 3 per cent). In one instance there was a positive reaction to 0.00002 mg. purified protein derivative (PPD) at 48 and 72 hours. In another, a first-strength test of 1 to 1000 Old Tuberculin was negative, but a month later a second-strength test was positive. At this time an emulsion from an affected lymph node was injected into guinea pigs with subsequent recovery of acid-fast bacilli. Histologically, this showed a developing tuberculosis superimposed on a background of sarcoidosis. This case is one of those included in the group positive for acid-fast organisms. The third case, autopsied Case 14, showed a reversal of the tuberculin test from negative to positive subsequent to the development of tuberculous meningitis.

Wassermann or Kahn tests. Wassermann or Kahn tests were reported in 151 patients, with 140 giving negative reactions. Eight (about 5 per cent) gave positive reactions, and 3 both negative and positive results. These figures are compatible with prevalence rates of syphilis for the general population.

Sedimentation rate. The sedimentation rate of red blood cells (Cutler technic) was determined in 52 patients; in 20 (38 per cent) the rate was normal (0 to 10 mm. in one hour); in 32 (62 per cent), the rate equaled or exceeded 11 mm. in one hour; in 14 of the latter (27 per cent of the total number tested) the rate exceeded 30 mm.

Plasma proteins. In nearly one-third of the 40 patients in whom the total plasma protein was determined, the value was 8 grams or higher for each 100 ml. of blood. Both albumin and globulin fractions were recorded in 31 instances and 19 (over 60 per cent), the globulin was 3.0 grams or higher for each 100 ml. of blood. The relationship of the albumin and globulin fractions to the total protein is shown graphically in Figure 2.

RESULTS OF THE QUESTIONNAIRE

Questionnaires were sent to 114 of the patients and 67 replies were returned by the date of termination of this study. A summary of the answers to the most important questions revealed that: (1) All the patients were still living, although 50 reported recurrence of symptoms referable to sarcoidosis; (2) 42 had reported to hospitals for medical care since the date of the original diagnosis; (3) most of them had no opinion as to the possible cause of their disease.

No indication as to epidemiologic association of cases was obtained from answers to the question as to the existence of the same disease in other persons associated with them at the time of the onset of symptoms. Sixty-three knew of no others who were ill, and the remaining 4 each reported the illness of one other person. It is questionable, however, whether the reported illness in these 4 was sarcoidosis, since the records at the Institute give no evidence of other instances of the disease in the immediate areas at the time these patients were hospitalized.

* Two other patients were considered to have tuberculosis on anatomic evidence without bacteriologic confirmation. (See autopsied Cases 10 and 11.)

The symptoms reported among the 50 patients who had symptoms were chest pain (17), enlarged lymph nodes (12), malaise (10), weakness (9), eye affections (8), cough (3), low-grade fever (2), skin manifestations (2), and fainting (1).

Thirty-five of the 67 men who replied had no opinion as to the possible cause of illness; of the remaining 22, 15 regarded exposure as the immediate cause and 6, over-exertion. Other causes assigned were colds, pneumonia, and tonsillitis (5); accidents (4); and inhalation of dust, dirt or smoke (2).

In response to a question as to other illnesses during the five years preceding hospitalization and biopsy, 53 patients reported none; 7, such infectious and

GLOBULIN AND ALBUMIN FRACTIONS OF PLASMA PROTEINS IN 31 CASES OF SARCOIDOSIS

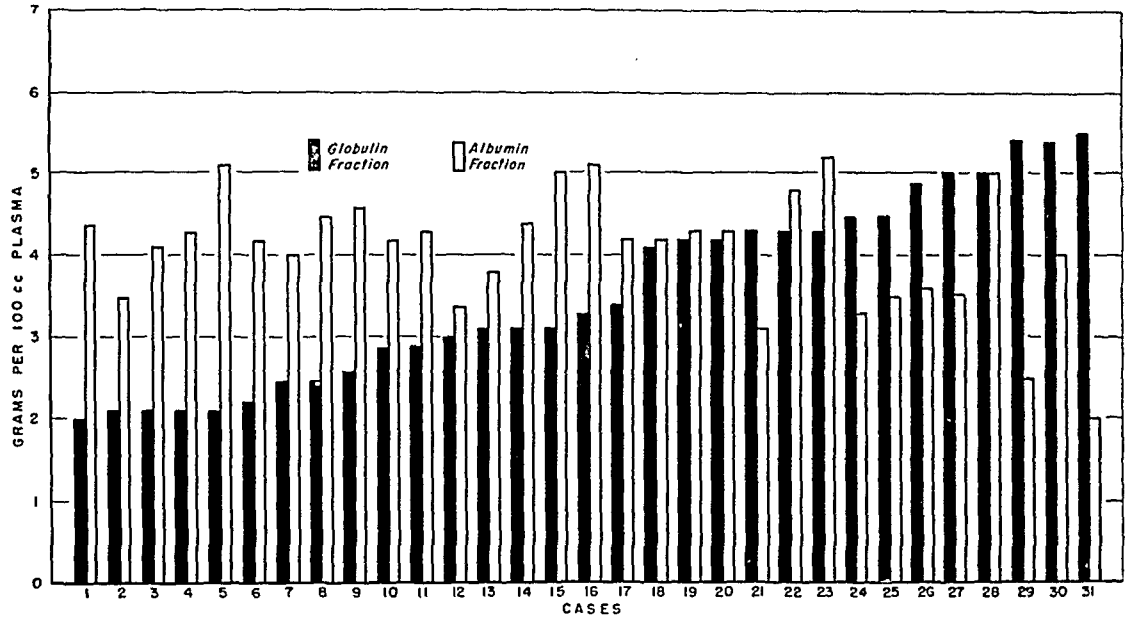


FIG. 2

parasitic diseases as pneumonia, measles, mumps, and hookworm; and 6, respiratory diseases, chiefly tonsillitis, "throat trouble", and "chronic cold". There was no reference to tuberculosis or cardiovascular disturbances in any reply.

ORGAN INVOLVEMENT

Two hundred forty-eight patients were found to have enlarged lymph nodes of whom 129, or more than 50 per cent, presented no other evidence of sarcoidosis. Generalized lymphadenopathy was present in 27 patients, or 11 per cent; in the remaining 102 patients, the cervical, axillary, and inguinal nodes, in that order of frequency, were the nodes primarily involved.

Organs other than lymph nodes affected in sarcoidosis are indicated in Table 5. In order of relative frequency, these were the lungs, skin, and bone. The eyes, which came next in this listing, were involved in less than 10 per cent of the

TABLE 6

FINDINGS IN 22 AUTOPSIED CASES OF SARCOIDOSIS

CASE NO., INITIALS	AIP ACC. NO.	AGE	RACE	SEX	CAUSE OF DEATH	ORGANS WITH SARCOID INVOLVEMENT	SYMPTOMS AND FINDINGS REFERABLE TO SARCOIDOSIS
1. HK	74926	62	W	F	Intestinal obstruction	Lungs, lymph nodes, liver, spleen	Splenomegaly; generalized lymphadenopathy Bilateral mottling of lungs on x-ray; tuberculin tests negative
2. VCG	85448	28	W	M	Gunshot wound	Heart, lung, lymph nodes, liver, spleen	No pertinent symptoms noted X-rays and tuberculin tests not done
3. WM	87717	34	N	M	Stab wound	Lungs, lymph nodes, liver, spleen	No previous history available
4. KBM	96016	26	N	M	Pulmonary sarcoidosis with right ventricular hypertrophy and failure	Lungs, lymph nodes, liver, spleen	Shortness of breath, cough, cyanosis Smears and cultures for bacteria and fungi negative X-ray: diffuse mottled infiltrations in both lungs
5. GWG	106812	22	N	M	Pupura (allergic ?)	Lungs, lymph nodes, liver, spleen	None
6. SOW	106987	26	W	M	Generalized peritonitis	Spleen	None Biopsy of ruptured spleen showed epithelioid tubercles; autopsy showed no remaining sarcoid
7. TBR	109562	Unknown	W	M	Acute gangrenous appendicitis	Lungs, liver, spleen	None
8. EC	113762	20	W	M	Accidental shell explosion	Lungs, lymph nodes, liver	No record of previous illness
9. EH	115945	22	N	M	Renal vascular disease	Lungs, lymph nodes, liver, kidneys, brain, meninges	Epistaxis and chronic nasal obstruction, 1942; chest pain and cough, 1944 Sarcoidosis on biopsy of cervical lymph node Tuberculin tests negative Serum globulin 3.0 to 4.2 Gm. Acid-fast bacilli recovered on guinea pig inoculation of emulsion of postmortem lymph node

TABLE 6—*Continued*

CASE NO., INITIALS	AIP ACC. NO.	AGE	RACE	SEX	CAUSE OF DEATH	ORGANS WITH SARCOID INVOLVEMENT	SYMPTOMS AND FINDINGS REFERABLE TO SARCOIDOSIS
10. RK	119987	30	W	M	Addison's disease	Lungs, lymph nodes, liver, spleen, kidneys, bone marrow	Weight loss, fatigue, low grade afternoon fever, progressive weakness Sarcoidosis on biopsy of inguinal lymph node Guinea pig inoculation negative Serum globulin 4.6 Gm.
11. RRR	121747	54	W	M	Addison's disease	Lungs, lymph nodes, heart, liver, spleen, kidneys, skeletal muscle	Weakness, loss of weight: hepatosplenomegaly and hyperproteinemia No tuberculin skin tests done Kahn tests repeatedly doubtful
12. TLD	124086	Unknown	W	M	Fat embolism due to trauma	Lung	None
13. ZGK	134190	33	N	M	Knife wound, abdomen	Liver, spleen	None
14. OC	148970	30	N	M	Tuberculous meningitis	Lungs, lymph nodes	Weakness, enlarged lymph nodes Sarcoidosis diagnosed on biopsy of two lymph nodes Tuberculin tests repeatedly negative for 6 months, became positive just prior to development of tuberculous meningitis
15. MW	149058	22	W	M	Pulmonary hemorrhage (due to sarcoidosis ?)	Lungs, lymph nodes, liver, spleen	Cough, one month's duration
16. NM	150321	25	N	M	Generalized peritonitis	Lungs, lymph nodes, liver, spleen, kidneys, small intestine	None
17. MC	151036	35	N	M	Sarcoidosis of central nervous system	Brain, meninges, lungs, lymph nodes, liver, spleen	"Spells" lasting 30-60 minutes during a 3 month period Progressive weakness, lethargy, partial deafness, followed by epileptiform attack
18 KS	167488	32	W	M	Gunshot wound, chest	Spleen	No previous history available

TABLE 6—*Continued*

CASE NO., INITIALS	AIP ACC. NO.	AGE	RACE	SEX	CAUSE OF DEATH	ORGANS WITH SARCOID INVOLVEMENT	SYMPTOMS AND FINDINGS REFERABLE TO SARCOIDOSIS
19. GKR	169668	38	W	M	Suicide	Paratracheal lymph nodes, liver, spleen	None Patient under observation for mental disorder
20. EB	172709	25	N	M	Sarcoidosis of myocardium	Pituitary, thyroid, lungs, lymph nodes, heart, liver, spleen, pancreas, kidneys, prostate, epididymis, brain, meninges, retina, skeletal muscle	Visual disturbances Enlarged mediastinal lymph nodes, pulmonary infiltration on x-ray Biopsy: sarcoidosis of epitrochlear and inguinal nodes Ventricular fibrillation terminally
21. JRM	173430	18	W	M	Cholecystectomy, operative death	Lymph nodes, liver	Reversal of albumin-globulin ratio with hyperglobulinemia
22. RJ	163480	26	N	M	Pulmonary tuberculosis	Lungs, lymph nodes, liver, spleen	Cough, fever, weight loss Diffuse mottled infiltration of lungs on x-ray Sputum negative, later positive Albumin-globulin ratio normal Sarcoidosis diagnosed at autopsy

Case 1: AIP Acc. 74926. A 62 year old white woman was first examined in 1938. Roentgenograms showed diffuse bilateral mottling of both lung fields. One year later definite splenomegaly was detected and this was followed by generalized superficial lymphadenopathy. Tuberculin tests were negative. The patient was without symptoms until her rather sudden death in January, 1940. At autopsy the cause of death was found to be intestinal obstruction due to pre-existing peritoneal adhesions; sarcoid granulomata were observed in the lungs, lymph nodes, liver, and spleen.

Case 2: AIP Acc. 85448. A 28 year old white man committed suicide by shooting himself in the head. He had been hospitalized 4 times during the previous year, the diagnosis in each instance being "constitutional psychopathic state; emotional instability". Physical examinations were consistently negative. Although death resulted from the gunshot wound of the head, autopsy revealed disseminated sarcoidosis in heart, lung, lymph nodes, liver and spleen.

Case 3: AIP Acc. 87717. A 34 year old Negro man was stabbed to death. No information was available as to the previous history. At autopsy, in addition to the fatal hemorrhage secondary to severance of the left brachial artery, multiple sarcoid granulomata were found in lungs, lymph nodes, liver and spleen. A healed primary tuberculous complex was encountered in the mediastinal lymph nodes.

Case 4: AIP Acc. 96016. A 26 year old Negro man was admitted to the hospital July 12, 1942, complaining of shortness of breath and cough of one month's duration. The symptoms increased in severity, and by December 1942, cough was almost continuous. Cyanosis

developed, and oxygen was administered at frequent intervals during the next five months until his death on May 29, 1943. During this illness roentgenograms showed diffuse, mottled infiltrations throughout both lung fields, but all attempts to isolate bacteria or fungi by smear or culture failed. Autopsy revealed sarcoidosis involving lungs, lymph nodes, liver and spleen. Right ventricular hypertrophy secondary to the pulmonary involvement was advanced, the right ventricle measuring as much as 1.2 cm. in thickness.

Case 5: AIP Acc. 106812. A 22 year old Negro man was hospitalized on January 20, 1944, because of epistaxis of two days' duration. He had been a steel worker in civilian life, and had had frequent nosebleeds which usually stopped after a few hours. Recurrence of bleeding on the day after admission was followed by coma on January 22. Plasma infusion and vitamin K and C therapy reduced nasal bleeding, but pulmonary edema developed and death ensued. Formed blood elements were within normal values. Autopsy showed petechiae in all tissues and a large subarachnoidal hemorrhage. Since no cause could be demonstrated for the bleeding tendency, either clinically or pathologically, it was attributed to an allergic state. In addition, sarcoid granulomata were present in lungs, lymph nodes, liver and spleen, but this condition was not regarded as being related to the purpura.

Case 6: AIP Acc. 106987. A 26 year old white man was admitted to the hospital on January 2, 1944, following an automobile accident. A ruptured spleen was removed at operation. The surgical specimen showed innumerable epithelioid tubercles, sharply outlined, usually with a fine collagen band. The few necrotic areas in the centers of the tubercles showed a "fibrinoid" character. The diagnosis of sarcoidosis of the spleen was made. The patient died 10 months later of peritonitis secondary to acute gangrenous appendicitis. At autopsy the tissues revealed no remaining evidence of sarcoidosis. (It should be mentioned, however, that study of the lymphatic tissues was not done at autopsy, because the pathologist was not aware of the earlier diagnosis.)

Case 7: AIP Acc. 109562. A white man, age not stated, was admitted to a hospital in the Asiatic Theater on June 8, 1943, with generalized abdominal cramps and tenderness. At operation a ruptured appendix was removed. He died almost immediately, his temperature having rapidly reached 109°F. In addition to the abdominal lesion, autopsy revealed miliary granulomata, characteristic of sarcoidosis, in the lungs, liver and spleen.

Case 8: AIP Acc. 113763. A 20 year old white man was one of 7 killed instantly in an accident on a firing range. He had had no previous illness, according to his records. Autopsy disclosed sarcoidosis of lungs, lymph nodes and liver.

Case 9: AIP Acc. 115945. A 22 year old Negro man was first hospitalized in North Africa in 1942 because of frequent nosebleeds, chronic obstruction of the left naris, and severe frontal headaches. Chest pain and cough led to his readmission in July, 1944. He was evacuated to a hospital in the United States, where a cervical lymph note was removed and the diagnosis of sarcoidosis was made on biopsy. Urinary abnormalities gradually appeared along with a rising blood pressure, and the patient died in azotemia on April 27, 1945, nearly three years after he had first been admitted to the hospital. The patient's tuberculin reactions had consistently been negative and the serum globulin had been evaluated to 3.0 to 4.2 Gm. per 100 ml. of blood. At autopsy, arteritic and periarteritic lesions of the small and medium-sized arteries accounted for the renal failure. Sarcoidosis involved the lungs, lymph nodes, liver, kidneys, brain and meninges. A lymph node emulsion was injected into guinea pigs. These animals, which died in an enteritis epidemic affecting nearly the entire animal colony, showed small necrotic lesions in spleen and liver which harbored acid-fast bacilli.

Case 10: AIP Acc. 119987. A 30 year old white man was hospitalized May 3, 1944, because of weight loss, fatigue and daily afternoon fever of low grade. Biopsy revealed the characteristic pattern of sarcoidosis in a reactive inguinal lymph node. Guinea pig inoculation of the emulsified lymph node was negative. Serum globulin was 4.6 Gm. per 100 ml. of blood. Weakness was progressive and the patient finally died October 15, 1944, while on leave from the hospital. Autopsy disclosed extensive necrosis of both adrenal glands, grossly and microscopically resembling old caseous tuberculosis. The typical discrete granulomas of

sarcoidosis were found in lungs, lymph nodes, liver, spleen, kidneys and bone marrow. In none of the lesions, including those of the adrenal glands, could acid-fast bacilli be found.

Case 11: AIP Acc. 121747. A 54 year old white soldier with 31 years' service, had been admitted to the hospital 3 times in the two years preceding his terminal illness. Pertinent findings had included weakness, loss of weight and hypotension, as well as hepatosplenomegaly and hyperproteinemia. Unfortunately, no tuberculin skin test was done; the Kahn test was repeatedly doubtful. After he had been admitted to the hospital for the last time, the patient continued to lose weight. Two days before his death he vomited coffee-ground material and lapsed into coma. Autopsy disclosed complete destruction of adrenal parenchyma with replacement by amorphous and granulomatous tissue somewhat resembling that of old, caseous tuberculosis. Small foci of tuberculous reinfection were present in the pulmonary apices bilaterally. In addition, a branch of the superior pancreaticoduodenal artery had been eroded by a small peptic ulcer. Distinct from the tuberculous lesions, grossly and microscopically, typical sarcoid granulomas were found densely "seeded" in lungs, lymph nodes, heart, liver, spleen, kidneys and skeletal muscle. Exhaustive bacteriologic studies were carried out on all the lesions, including smears, cultures and guinea pig inoculations. Two acid-fast rods were found in material from the right pulmonary apex; all other smears, cultures and inoculations were negative.

Case 12: AIP Acc. 124086. A white man of unknown age was hospitalized May 23, 1944, with traumatic amputation of the right lower leg. The patient did not respond to sulfadiazine and blood transfusions and died on May 28. Autopsy disclosed systemic fat embolism with cerebral involvement and sarcoidosis of the lung.

Case 13: AIP Acc. 134190. A 33 year old Negro sustained a penetrating knife wound of the abdomen in an accident. He was removed immediately to a field hospital where he died within three hours. Autopsy disclosed that the duodenum and mesentery had been perforated. In addition, sarcoidosis was encountered in the liver and spleen.

Case 14: AIP Acc. 148970. A 30 year old Negro was hospitalized in January, 1945, because of weakness. Two lymph node biopsies were characteristic of sarcoidosis. Tuberculin tests were repeatedly negative. In July, six months later, there was sudden elevation in temperature, weight loss, and reversal of the tuberculin reaction to positive. By November signs of meningitis had developed and the spinal fluid contained acid-fast bacilli. The patient died of tuberculous meningitis December 16, 1945. At autopsy, tuberculosis of meninges, lungs, liver and spleen was found. In addition, lesions typical of sarcoidosis were encountered in lungs and lymph nodes, some of the giant cells in the tubercles containing "asteroids" and others, Schaumann bodies.

Case 15: AIP Acc. 149058. A 22 year old white man was admitted to the hospital November 4, 1944, with cough of one month's duration and a cold which had begun four days before. No information as to his previous history was available. He died on the second hospital day of a massive pulmonary hemorrhage. At autopsy, sarcoidosis was found involving the lungs, lymph nodes, liver and spleen. No definite cause could be found for the massive hemorrhage, although weakening of the vessel walls by the perivascular granulomatous reaction was considered a possibility by the pathologist.

Case 16: AIP Acc. 150321. A 25 year old Negro was hospitalized July 29, 1945, because of an epidermoid carcinoma originating in the skin of the right heel. In August a right mid-tibial amputation was performed. Subsequently metastatic carcinoma was found on removal of an inguinal lymph node. Apparent improvement was noticed, then the patient suddenly experienced abdominal pain and went into severe shock. Death occurred immediately thereafter, on November 23, 1945. Autopsy revealed generalized peritonitis resulting from perforation of a chronic duodenal ulcer. Unsuspected sarcoidosis involved the lungs, lymph nodes, liver, spleen, kidneys and small intestine.

Case 17: AIP Acc. 151036. A 35 year old Negro had been hospitalized prior to March 1945, with a diagnosis of "psychoneurosis, anxiety state, mild". The patient had apparently responded to encouragement, treatment with vitamins, placebos, and hypodermic injections

of sterile water. Information was elicited later of spells lasting 30 to 60 minutes, consisting of a "loss of comprehension of events", occurring about once a week since January 1945. On March 20 he fell down, was unable to walk, and a speech defect developed. He became lethargic, confused, hard of hearing, and unable to comprehend orders. Five days later he had an epileptiform attack after which he could be roused only with difficulty. No medication was given on the assumption that he was in postconvulsive somnolence. He was found dead in bed the next morning, March 26. Autopsy revealed characteristic healing and healed lesions of sarcoidosis in the brain, meninges, lungs, lymph nodes, liver and spleen. Special stains failed to disclose acid-fast bacilli in any of these organs. Sarcoidosis had not been suspected.

Case 18: AIP Acc. 167488. A 32 year old white man, a German prisoner of war, was killed by a bullet which punctured both lungs. No previous history was available. In addition to the traumatic changes, autopsy revealed sarcoidosis of the spleen.

Case 19: AIP Acc. 169668. A 38 year old white man was admitted to the hospital November 27, 1945, where the diagnosis made was "schizophrenia, simple type". He committed suicide by hanging on February 25, 1946, while in the hospital. Autopsy disclosed sarcoid lesions in the paratracheal lymph nodes, liver and spleen.

Case 20: AIP Acc. 172709. A 25 year old Negro man, weighing about 200 pounds, was admitted to the hospital in July 1945, because of redness, blurring and moderate loss of vision in both eyes. On readmission in November 1945, roentgenograms showed enlarged mediastinal lymph nodes and pulmonary infiltration in the form of small miliary nodules with increased linear markings. On January 11, 1946, some enlargement of right epitrochlear and right inguinal nodes was noted. Sarcoid was diagnosed on biopsy. He developed a rapid pulse rate of 160 per minute and it was believed that he had either a "fast fibrillation or a simple ventricular (ectopic) tachycardia." Rapid parenteral digitalization was started but was discontinued two days later because the symptoms had subsided. Relapse to ventricular tachycardia followed and he was then treated with oxygen. The patient improved, but again sudden ventricular fibrillation occurred and he died on February 18, 1946. No tuberculin skin tests or plasma protein determinations were made on this patient. Generalized sarcoidosis with extensive involvement of the myocardium was found at autopsy. The following organs showed sarcoid lesions: pituitary, thyroid, lungs, lymph nodes, heart, liver, spleen, pancreas, kidneys, prostate, epididymis, brain, meninges, retina and skeletal muscle.

Case 21: AIP Acc. 173430. An 18 year old Mexican was admitted to the hospital 3 times between August and December 1945, with jaundice and pain in the abdomen. On the third admission a diagnosis of cholecystic disease was made. After careful study during the next three months, operation was performed to remove the gallbladder. Auricular fibrillation developed while the patient was on the operating table and he died before he could be returned to the ward, March 28, 1946. During hospitalization the albumin-globulin ratio was always reversed, the serum globulin being 3.1 to 3.3 grams per 100 ml. of blood at various times. At autopsy, changes observed in the liver were consistent with those after recovery from epidemic hepatitis. Characteristic sarcoid granulomas were encountered in lymph nodes and liver.

Case 22: AIP Acc. 163480. A 26 year old Negro man entered the hospital in March 1945, with sudden fever. Roentgenograms showed mottled density of the upper half of the left lung and middle portion of the right lung. The albumin-globulin ratio was normal and roentgenologic examination of the small bones of the hands was negative. Sputum examinations, which had been repeatedly negative, became positive, and shortly before death his temperature rose to 104 F. He died September 9, 1945. Autopsy revealed chronic tuberculous pneumonitis with cavitation in all lobes of both lungs and miliary tuberculous lesions in liver, spleen, kidneys and testes. Distinct from these lesions, sarcoid tubercles were encountered in lungs, lymph nodes, liver and spleen, many of these granulomas showing giant cells containing Schaumann bodies.

cases. A striking racial difference appears, however, in the number of patients whose eyes were affected, since lesions in this location were observed in only 1 per cent of white patients in contrast to 14 per cent of the Negroes. Clinical diagnoses in these cases ranged from general statements as to eye involvement to diagnoses of nodular iritis (2 cases), iridocyclitis (2), secondary glaucoma (2), uveitis (1), and chorioretinitis (1). Sarcoidosis of other organs, liver, spleen, brain and meninges, kidneys and heart, was found at operation or autopsy.

AUTOPSY REPORTS

From the records in the literature, reports of 62 cases have been found in which postmortem examination was carried out. Rubin and Pinner[17] have compiled 43 cases occurring prior to 1944. Since then, or not included in that series, Reisner[15] has reported 3; Longcope[11] 4; while Skavlem and Rittenhof,[20] Enzer,[3] Friedman,[5] Lopes de Faria,[12] Johnson and Jason,[9] Pruvost *et al.*,[14] Crane and Zetlin,[2] Smith and Sauls,[21] Ronchese,[16] Thomas,[22] Katz *et al.*,[10] and Clearkin *et al.*[1] have reported single cases. The following 22 cases from the files of the Institute, which include records of autopsy, raise this total to 84.

DISCUSSION

Autopsied cases. It is of interest to note that sarcoidosis was unsuspected in 14 or nearly two-thirds (Cases 2, 3, 5, 7, 8, 11–13, 15–19, 21) of the 22 fatal cases. Indeed, in 5, or nearly one-fourth, death was violent and accidental or incidental to military activity, the victims having previously been in good health. This absence of symptoms is entirely in keeping with accepted concepts of the disease, although the percentage in this series is higher than in any other published series. This difference is probably best explained by the fact that the percentage of persons autopsied after traumatic death is higher in the Army than in civilian populations.

It will be further noted that of the 22 deaths only 3 (Cases 4, 17, 20) were unquestionably due to sarcoidosis, while in Case 15 the disease might possibly have been implicated. When calculated for the entire series of 300 cases, the mortality rate was 1.3 per cent.

Figure 3, listing the organs involved in number and percentage of cases, is fairly consistent with accepted figures. In 12 of the cases (over 58 per cent) the lungs, lymph nodes, liver and spleen were all involved. The percentage with lymph node involvement would probably have been higher than is indicated on the chart had a thorough investigation been made in each autopsy.

In Case 4 the extensive lung changes had resulted in severe damage to the right side of the heart as evidenced by both clinical and postmortem observations. The occurrence of clinical heart failure or pathologic dilation and hypertrophy of the right ventricle has been described several times,[10, 15, 18, 23] and has been thoroughly discussed by Longcope.[11] Direct cardiac involvement by sarcoid tubercles, causing death, as in Case 20, has been frequently mentioned in the literature,[3, 9, 15, 17, 21] as has asymptomatic cardiac sarcoidosis (Cases 2 and 11).

In three instances (Cases 8, 14, 22) there was associated proven tuberculosis.

In Case 14 a shift in tuberculin reactions from negative to positive occurred. The lesions of sarcoidosis in the lungs and lymph nodes in this case indicated that they were "older" than the distinguishable tuberculous ones. Likewise, in Case 22 the diseases were coexistent and recognizable microscopically. The presence of Schaumann bodies in the lesions of sarcoidosis in Case 22 was a positive help in differentiating the separate types of granulomas; for although they are not pathognomonic for sarcoidosis, they have not been observed in tuberculosis. In Case 9 the presence of tuberculosis was determined post mortem by inocula-

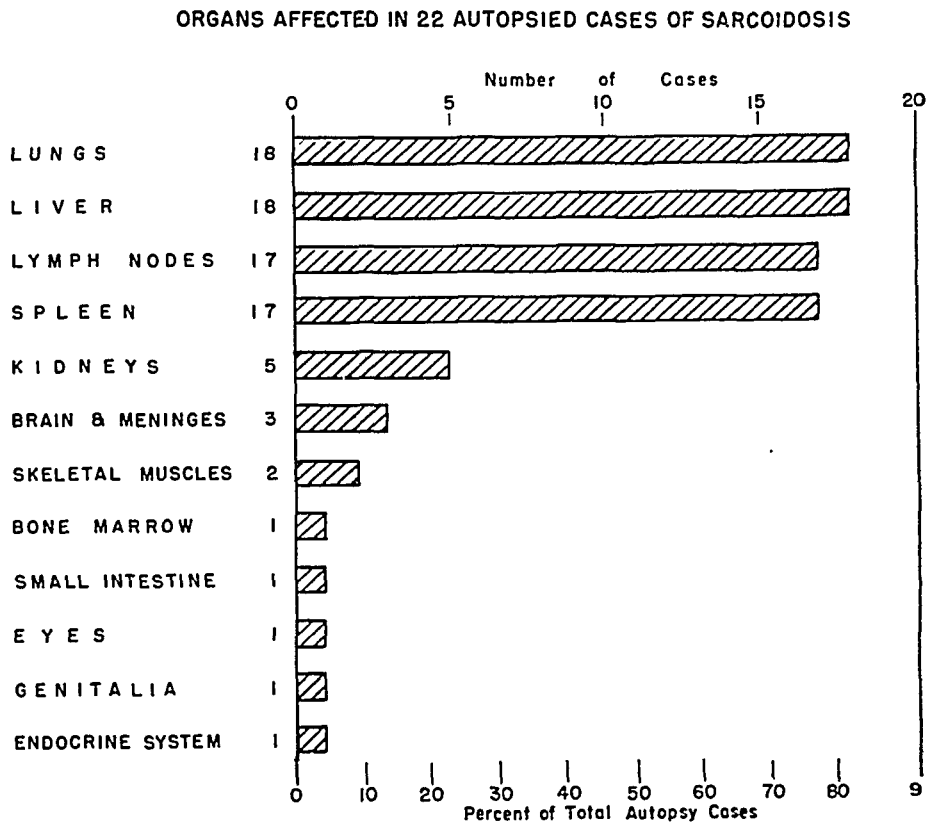


FIG. 3

tion of guinea pigs with lymph node emulsion. Some doubt could be cast upon the results because the animals died in an enteritis epidemic which swept away most of the colony. While it cannot be proved that both sarcoidosis and tuberculosis were present in Cases 14 and 22, this conclusion is permissible, especially in view of similar cases reported by Hollister and Harrell[8] in which they believed that the diseases were coexistent.

Case 5 in this series is similar to one reported by Enzer,[3] since in both instances the patients died of purpura. The mechanism of death in these two instances, however, may have been different; a background of allergy was postulated in our Case 5 in the absence of abnormalities of blood, i.e., thrombo-

cytopenia, while in Enzer's case the pancytopenia suggested hypersplenism. Certainly in both cases the occurrence of sarcoidosis was entirely incidental to the purpura.

Histopathology. In addition to those features enumerated in the opening paragraphs of this report under the heading Histologic Features, certain additional microscopic findings are of interest. A lesion which seemed to precede the development of sarcoid granulomas was occasionally encountered in uninvolved nodes adjacent to others containing well-defined sarcoidosis. This pre-sarcoid lesion consisted of a nonspecific proliferation of the littoral sinusoidal reticuloendothelium, giving the picture of wide, choked lymphatic sinosoids with little or no reaction in the rest of the node (Fig. 4A). The earliest granulomas regularly arose in juxtaposition to such a sinusoid (Fig. 4B), but attempts by serial section to show that these granulomas developed from the proliferated reticuloendothelium failed.

From its inception the sarcoid granuloma appeared to be a recognizable entity. The earliest lesions in this material were discrete epithelioid granulomas sharply delimited from the surrounding tissue and composed of uniform polyhedral cells with abundant, acidophilic cytoplasm, and large, ovoid and vesicular nuclei. A few lymphocytes may be seen within the nodule (Fig. 4B). A fully developed miliary granuloma presents a narrow rim of two or three layers of fibroblasts; the epithelioid cells elongate and tend to adopt a concentric pattern. Since the granulomas always develop in clusters, they may appear conglomerate. Usually, however, even in such a group the individuality of each one can be determined. The subsequent course is regression by fibrosis (Fig. 5A). At first the fibrosis occurs between and about the individual granulomas with compression and isolation of the lesions. The fibrous tissue then rapidly becomes compact, hyalinized, and ends as a dense scar in which but the barest suggestion of the previous nodule remains as a few pyknotic epithelioid cells, a small cluster of lymphocytes, or an isolated giant cell (Fig. 5B).

Frequently, all the granulomas in one organ are at approximately the same level of development or regression. This accounts for the peculiar monotony of the typical sarcoid pattern. Very occasionally the disease recurs in "crops",

FIG. 4A. Preceding the development of characteristic sarcoid granulomas, the lymph nodes show a pattern of dilated sinusoids and hyperplasia of the littoral reticulum of the sinuses. This process is particularly seen in lymph nodes adjacent to those already involved. × 100, AIP Neg. 97778.

FIG. 4B. The youngest granuloma of sarcoidosis arises frequently immediately adjacent to one of the dilated and "choked" sinusoids. Attempts to demonstrate origin of the epithelioid cells of the granuloma from the hyperplastic littoral reticulum cells by serial section were not successful. Hematoxylin and eosin stain, × 300, AIP Neg. 97692.

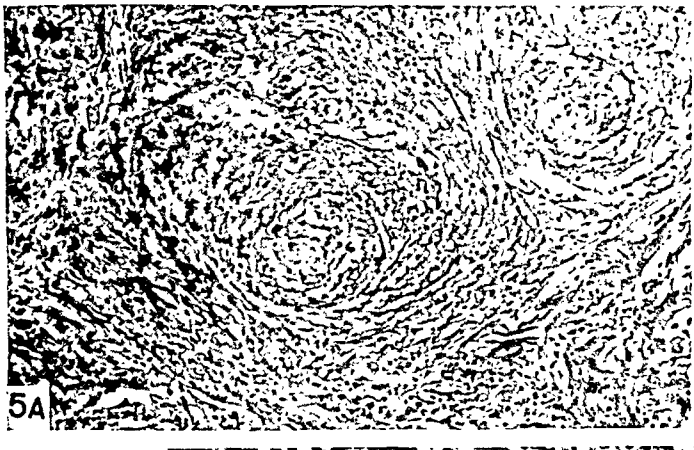
FIG. 5A. Fibrosis is proceeding in and about the granuloma of sarcoidosis, but the identity of the individual lesions remains. × 100, AIP Neg. 97638.

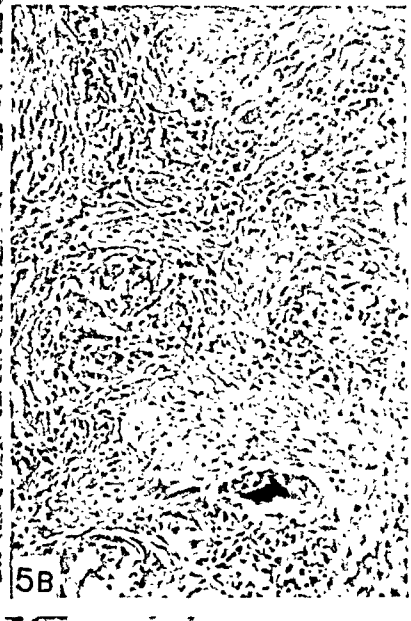
FIG. 5B. Healing has continued to nearly complete fibrosis leaving scattered giant cells, lymphocytes and a few pyknotic epithelioid cells. Hematoxylin and eosin stain, × 100, AIP Neg. 97780.

FIG. 6. The small granuloma shows a peripheral rim of two or three layers of fibroblasts from which fibrosis progresses. The process has advanced to show some degree of peripheral hyalinization at the periphery of this granuloma. Hematoxylin and eosin stain, × 300, AIP Neg. 97634.











FIGS. 4–6

with lesions of two, or possibly three, different ages. For any one "crop" the consistent pattern remains.

Giant cells have been frequently observed and described in the lesions of sarcoidosis. In this series the giant cells were occasionally of the Langhans type, although more often of the foreign body variety. This latter form is extremely variable, especially when the cell is accommodating to an irregular Schaumann body (Fig. 7). Nuclei number from 10 to 50 or more, retaining the ovoid vesicular appearance of the histiocytic nucleus. The cytoplasm is usually eosinophilic and granular, but often shows peculiar fine vacuolization which is positive to specific stain for fat.

Giant cell inclusions of two types are occasionally encountered in granulomas of sarcoidosis, the Schaumann body[19] (Fig. 7) and the spiculated body[24] or "asteroid"[5] (Fig. 8). Inclusions were encountered in 6 per cent (18 cases) in our histologic material: Schaumann bodies in 3.6 per cent and "asteroids" in 2 per cent. In one case both types of inclusion occurred. The "asteroids" were always found in giant cells; Schaumann bodies, on the other hand, often were extracellular, looking as though they had been extruded from an adjacent giant cell. Neither of the inclusion bodies is specific for sarcoidosis, the Schaumann body occurring in at least two other conditions, in the lymph nodes of lymphopathia vereream and in giant cells in a lymph node immediately draining an area of regional ileitis (Fig. 9). The "asteroids" have been seen in a variety of chronic granulomatous diseases, including tuberculosis, leprosy, histoplasmosis, schistosomiasis and lipoidal granuloma. In fact, the "asteroid" occurs so much more frequently in lesions other than sarcoidosis, that one is inclined to be cautious of a diagnosis of sarcoidosis in "asteroid"-containing material.

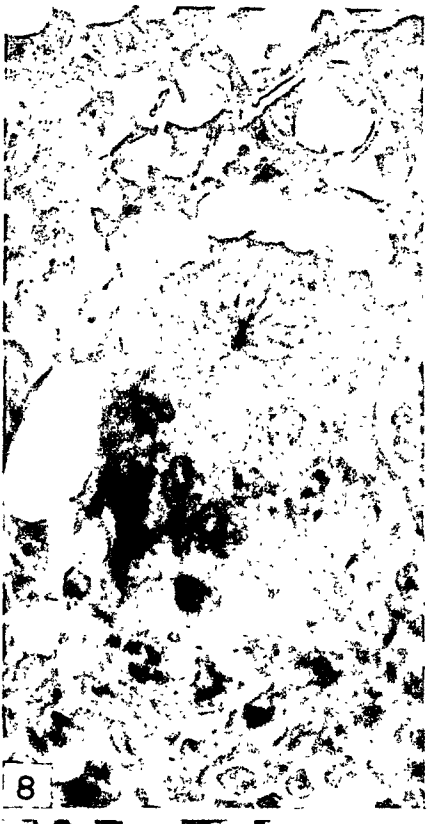
In an occasional case the presence of vacuolization had been observed both in the epithelioid cells comprising the granulomas and in the giant cells. In the latter the vacuolization is usually very fine, best described as dust-like, occurring in a peculiar ring occupying the center of the giant cell. The ordinary fat stains are positive in these cases, demonstrating the very fine fat droplets. As in the case of the inclusion bodies, these fat changes are by no means specific for sarcoidosis and occur in any of the lesions in which "asteroids" occur. The two findings, fine fat vacuolization and "asteroids", are coincidental so regularly that we feel there is a relationship between the two even though it has been impossible to demonstrate beyond question the transition of the one form into the other.

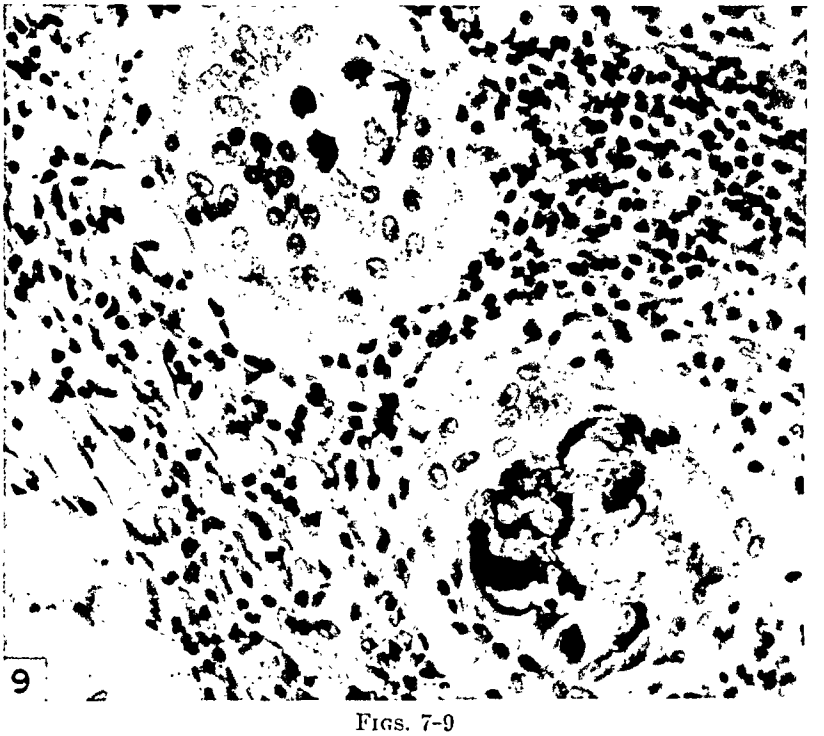
FIG. 7. A foreign-body giant cell containing about 20 recognizable nuclei is accommodating to a large Schaumann body. The typical concentric laminations and deep basophilia are shown. These bodies were found in 4 per cent of the cases of sarcoidosis. Hematoxylin and eosin stain, × 1000, AIP Neg. 95620.

FIG. 8. A spiculated body or "asteroid" is seen in a giant cell in sarcoidosis. The typical spider-like processes arise from a central eosinophilic body. These inclusions occurred in 2 per cent of the sarcoidosis material but much more frequently in a large number of other types of granulomas. Both types of inclusions, the spiculated body and the Schaumann body, are nonspecific. Hematoxylin and eosin stain, × 915, AIP Neg. 68312.

FIG. 9. Schaumann bodies in isolated foreign-body giant cells in a mesenteric lymph node adjacent to an area of regional ileitis. These inclusions are identical to those seen in sarcoidosis. Hematoxylin and eosin stain, × 355, AIP Neg. 97788.







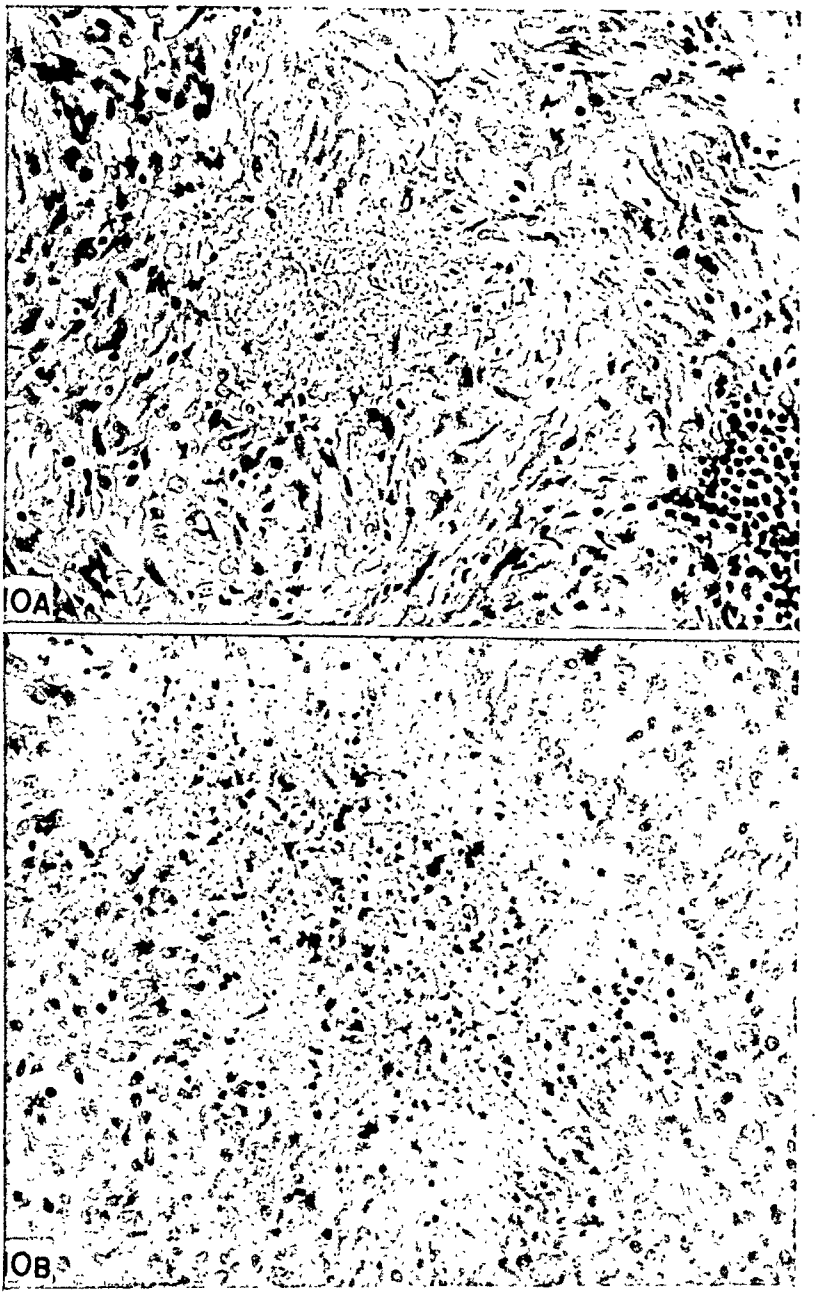
FIGS. 7-9

Necrosis in sarcoidosis has been infrequently described. In our material, varying amounts of necrosis have been encountered in 107 cases, or in 35 per cent of the total. The necrosis is characteristic in type and in location. It is invariably of the type referred to as "fibrinoid". Tinctorially it is strongly eosinophilic. Minute areas may be seen in the center of an individual granuloma, but the greatest degree of necrosis is in the area of confluence of several contiguous granulomas. A few intact lymphocytes may occur about the periphery of the necrotic area, but otherwise the necrosis is characteristically acellular (Fig. 10A). It is in the total lack of cells and cellular debris with homogeneous, fibrillar zones of necrosis that this type is distinguished from the caseation of tuberculosis (Fig. 10B).

SUMMARY AND CONCLUSIONS

This clinical and pathologic review of 300 cases with granulomas diagnosed as sarcoidosis includes brief reports of 22 autopsied cases. All lesions conformed to histologic criteria for the disease.

1. *Clinical findings.* The review of clinical features showed the following:
 a. The disease reached peak occurrence in the 20–24 year age group.
 b. The disease occurred 17 times as often among Negro men as among white men in the Army.
 c. Of the 258 patients who presented symptoms on admission to the hospital, the most common were: enlarged lymph nodes (86 per cent), cough (20 per cent), weight loss (19 per cent), skin manifestations (16 per cent), low-grade fever (15 per cent), malaise (10 per cent), and weakness (10 per cent).
 d. In the entire group of 300 patients, the organs most frequently affected were: lymph nodes, 83 per cent; skin, 21 per cent; bone, 17 per cent; eyes and liver, each 9 per cent; and spleen, 8 per cent. Other organs were each involved in less than 3 per cent of the cases.
 e. Over two-thirds of the patients were admitted to hospitals within six months after they first noticed symptoms.
 f. Roentgenologic examinations showed mediastinal widening in 76 per cent of patients examined, while small bone involvement was seen in 36 per cent of the patients who had roentgenologic examinations of the extremities.
 g. Tuberculin skin tests were negative in 97 per cent of 88 patients.
 h. The sedimentation rate was elevated in 62 per cent of 52 patients.
 i. Hyperproteinemia of 8 Gm. or more per 100 ml. of blood was present in one-third of 40 patients.
2. *Follow-up.* Seventy-five per cent of the patients answering a follow-up questionnaire reported recurrence of symptoms consistent with sarcoidosis; 94 per cent knew of no other similar cases occurring at the time and place as theirs, and the replies of the other 4 (6 per cent), each of whom reported one other similar illness, can be seriously questioned. As to cause, the



FIGS. 10A AND 10B. A and B contrast the fibrillar "fibrinoid" necrosis, A, of sarcoidosis and very early caseation necrosis, B, of tuberculosis. In sarcoidosis acellularity and lack of nuclear debris is noted, while in early tuberculous necrosis degenerating cells and cell fragments make up the necrotic zone. Hematoxylin and eosin stain, × 300, AIP Negs. 97646 and 97696.

majority did not know the cause, and most of the remaining persons attributed their trouble to exposure, overexertion, or upper respiratory infections.

3. *Autopsied cases.* Twenty-two autopsied cases of sarcoidosis are added to 62 collected from the literature. Sarcoidosis was unsuspected until autopsy in 14, or in nearly two-thirds of our cases, and in only 3 was death unequivocally due to sarcoidosis. Three of the 22 patients had associated sarcoidosis and tuberculosis (2 from whom tissue had been removed at operation also showed associated tuberculosis) and an additional 2 of the 22 showed abacterial, caseating lesions of the adrenal glands with clinical Addison's disease.
4. *Histopathology.* Review of the histopathologic material showed the following:
 a. In lymph nodes, proliferation of the sinusoidal reticulum almost invariably preceded the development of the tubercles of sarcoidosis.
 b. The miliary granuloma of sarcoidosis was characteristic and in its adult form appeared as a discrete, sharply delimited lesion, composed of concentrically arranged, variably elongated, epithelioid cells. Since all the granulomas in one organ were usually at the same stage of development, a peculiar monotony of pattern resulted.
 c. The lesions of sarcoidosis healed by fibrosis.
 d. Giant cells of both Langhans and foreign-body type were common.
 e. Giant cell inclusions occurred in 6 per cent of the material, Schaumann bodies in 4 per cent and spiculated bodies ("asteroids") in 2 per cent. None of these was specific for sarcoidosis.
 f. "Fibrinoid" necrosis, usually distinguishable from the caseation of tuberculosis, occurred in 35 per cent of this material.

REFERENCES

1. Clearkin, P. A., Bettencourt-Gomes, S. C., and Bornstein, A.: Pulmonary sarcoidosis; report of a case. Caribbean M. J., **7:** 13–18, 1945.
2. Crane, A. R., and Zetlin, A. M.: Hemolytic anemia, hyperglobulinemia and Boeck's sarcoid. Ann. Int. Med., **23:** 882–889, 1945.
3. Enzer, N.: Generalized Boeck's sarcoidosis with thrombocytopenic purpura; a report from the proceedings of the American Association of Pathologists and Bacteriologists. Am. J. Path., **22:** 663–664, 1946.
4. Freiman, D. G.: Sarcoidosis. New England J. Med., **239:** 664–671, 709–716, 743–749, 1948.
5. Friedman, M.: Sarcoidosis of the spleen; report of a case with autopsy and a study of intracellular "asteroid bodies". Am. J. Path., **20:** 621–635, 1944.
6. Hagemann, P. K. H.: Fluoreszenzfärbung von Tuberkelbakterien mit Auramin. München. med. Wchnschr., **85:** 1066–1068, 1938.
7. Harrell, G. T.: Generalized sarcoidosis of Boeck; a clinical review of 11 cases, with studies of the blood and etiologic factors. Arch. Int. Med., **65:** 1003–1034, 1940.
8. Hollister, W. F., and Harrell, G. T.: Generalized sarcoidosis of Boeck accompanied with tuberculosis and streptococcic bacteremia; clinico-pathologic study with autopsy and animal inoculations. Arch. Path., **31:** 178–188, 1941.
9. Johnson, J. B., and Jason, R. S.: Sarcoidosis of the heart; report of a case and review of the literature. Am. Heart J., **27:** 246–258, 1944.
10. Katz, S., Cake, C. P., and Reed, H. R.: Sarcoidosis. New England J. Med., **229:** 498–508, 1943.
11. Longcope, W. T.: Sarcoidosis or Besnier-Boeck-Schaumann disease. J. A. M. A., **117:** 1321–1327, 1941.

12. Lopes de Faria, J.: Tuberculose não caseosa (sarcoide de Boeck), tuberculose caseosa e lepra lepromatosa, caso de necropsia. Rev. brasil. de leprol., **10:** 405–418, 1942.
13. Pinner, M.: Noncaseating tuberculosis; an analysis of the literature. Am. Rev. Tuberc., **37:** 690–728, 1938.
14. Pruvost, P., Hautefeuille, E., Canetti, G., and Mabileau, J.: Maladie de Besnier Boeck-Schaumann et tuberculose. Arch. méd.-chir. de l'app. respir., **14:** 405–430, 1939–41.
15. Reisner, D.: Boeck's sarcoid and systemic sarcoidosis (Besnier-Boeck-Schaumann disease); study of 35 cases. Am. Rev. Tuberc., **49:** 289–307 and 437–462, 1944.
16. Ronchese, F.: Sarcoid and tuberculosis; report of a case with autopsy. Arch. Dermat. and Syph., **46:** 860–871, 1942.
17. Rubin, E. H., and Pinner, M.: Sarcoidosis; one case report and literature review of autopsied cases. Am. Rev. Tuberc., **49:** 146–169, 1944.
18. Schaumann, J.: Lymphogranulomatosis benigna in the light of prolonged clinical observations and autopsy findings. Brit. J. Dermat., **48:** 399–446, 1936.
19. Schaumann, J.: On the nature of certain peculiar corpuscles present in the tissue of lymphogranulomatosus benigna. Acta med. Scandinav., **106:** 239–253, 1941.
20. Skavlem, J. H., and Ritterhof, R. J.: Coexistent pulmonary asbestosis and sarcoidosis. Am. J. Path., **22:** 493–517, 1946.
21. Smith, C., and Sauls, H. C.: Sarcoidosis; an incidental finding in the postmortem examination of an 81 year old woman dying of coronary occlusion. J. M. A. Georgia, **34:** 87–91, 1945.
22. Thomas, C. C.: Sarcoidosis. Arch. Dermat. and Syph., **47:** 58–73, 1943.
23. Tice, F., and Sweany, H. C.: A fatal case of Besnier-Boeck-Schaumann's disease with autopsy findings. Ann. Int. Med., **15:** 597–609, 1941.
24. Wohlback, S. B.: A new type of cell inclusion, not parasitic, associated with disseminated granulomatous lesions. J. M. Research, **24:** 243–257, 1911.

Acknowledgment. The authors are indebted to Walter Z. Zwergel, Research Assistant at the Army Institute of Pathology, for aid in assembling and preparing statistical material used in this paper.

HASHIMOTO'S AND RIEDEL'S STRUMAS AND LYMPHADENOID GOITER*

S. M. RABSON, M.D., AND J. E. ARATA, M.D.†

From the Department of Pathology, Saint Joseph Hospital, Fort Wayne, Indiana

Three cases, including that of a young man who had two operations within nine months, are the basis for this report which may shed light on the pathology and inter-relationship of Riedel's and Hashimoto's strumas and lymphadenoid goiter. The views expressed here tend to support those offered by Shaw and Smith,[23] Boyden, Coller and Bugher,[3] and Vaux.[24]

DISCUSSION OF TERMINOLOGY

Struma fibrosa (Riedel). In 1896 Riedel[20] described, unfortunately too briefly, a unique process in the thyroid gland which led to an extraordinarily hard tumor firmly bound to the surrounding structures. The large blood vessels of the neck and trachea could not be separated from the mass which suggested an inoperable new growth, whereas microscopic examination demonstrated infiltration and compression of thyroid tissues by "round cells". In each of the 2 reported cases operation had to be discontinued without removing the entire lesion. The first patient was entirely freed of his complaints but suffered repeated "strokes" and, 15 months after operation, died of nephritis. The other patient also recovered from the operation but expired two months afterward, apparently of pulmonary embolism. Necropsy was not done.

At the same meeting at which Riedel discussed the condition, Kardua[11] told of a "similar case" in a 12 year old girl in whom it was not possible to resect the gland completely. Eighteen months later the child had completely recovered.

In 1897 Riedel[21] reported another case, that of a 29 year old man with a similar illness. Treatment with potassium iodide had been futile, and at operation the surgeon was unable to remove the thyroid completely. He did excise a hard, gray to white walnut-sized portion. Microscopically, there were spindle and round cells, and it was difficult to decide whether the process was inflammatory or neoplastic, but further clinical observation established its benign character. Six months later, Riedel removed a wedge-shaped area to relieve pressure that was causing nocturnal dyspnea. Hoarseness continued, but respiratory embarrassment ceased. Following operation the mass became smaller.

In 1910 Riedel[22] reported upon the subsequent history of 2 of the patients. The first patient mentioned in the report of 1896 had expired one year and three months after operation from nephritis: at autopsy the thyroid showed recession of the "iron-hard" struma. The patient mentioned in the 1897 report had no

* Read by title at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 14, 1948.

Received for publication, December 20, 1948.

† Present address: Mayo Clinic, Rochester, Minnesota.

complaints in 1901 referable to the thyroid and had been able to work. In 1905 he suffered a gastric hemorrhage and later that year dyspnea returned. From 1905 to 1909 the respiratory difficulty was so severe as to interfere with sleep. After that period the symptom gradually diminished in severity, and by 1910 there was no evidence of goiter. Laryngeal examination at that time revealed stenosis of the subglottic space and of the trachea.

The struma which Riedel described was typically seen in young persons, was characterized by subacute swelling and hardness of the thyroid and later was associated with marked dyspnea but no fever; microscopically, there was piling up of spindle and round cells between the normal elements of the thyroid, with progressive encroachment upon the latter, and "slight endarteritis, as noted in many chronic inflammatory processes". Riedel emphasized the infrequency of the disease, having seen only three instances among 1063 operations for goiter. He also pointed out that in Gilatscheck's patient the condition had spontaneously healed. Riedel had found hardening of the thyroid gland by calcification and in malignant disease, and stated that "iron-hard" struma was one of many thyroid conditions which might develop to a certain point and then recede. It was his opinion that many patients had died because of an erroneous diagnosis of inoperable carcinoma of the thyroid in which no attempt was made to relieve the pressure.

In none of Riedel's publications was there any mention of hyperthroidism or hypothyroidism, or the status of lymphoid tissues. He did not say what he meant by "round cells". Dyspnea apparently was the prominent symptom, and the operative findings were characteristic in that the thyroid gland was very hard, fixed to the surrounding structures, and virtually impossible to resect.

Struma lymphomatosa (Hashimoto). In 1912, two years after Riedel's last communication, Hashimoto[7] described in detail "lymphomatous changes of the thyroid (struma lymphomatosa)". Much confusion and misunderstanding have arisen, however, because the following important phrase in Hashimoto's prelude has been discounted or overlooked: "a definite alteration of the parenchyma *and* (our italics) of the interstitium".

All 4 of his patients were women, over 40 years of age, who resided in nongoitrous areas. They gave no history of severe pressure symptoms or of other thyroid disturbance. They all developed postoperative myxedema, requiring the administration of thyroid substance, and their convalescence was prolonged. Microscopically, the lymphoid follicles were richest in the border zone of the extirpated glandular tissues, and the acini were lined by a single layer of epithelial cells. The status of stroma and its relation to parenchyma were of especial interest, and Hashimoto's words are worth quoting.

"The character of the connective tissue is a very important one in our cases. It showed everywhere a marked proliferation which offered a very pretty picture with van Gieson's stain. The former [connective tissue] was diffuse, and in Case 4 was especially well pronounced so that the lobular arrangement of the acini was no longer recognizable. Also interesting was the relationship between the connective tissue proliferation and the change in the alveoli. The alveoli in the proliferated connective tissue were very sparse and were

characterized by the small size. They contained only little substance [colloid] and that little was represented by a small amount of amorphous, dirty red-staining material. In isolated places one sees a circumscribed strongly developed mass of connective tissue enclosing a number of very small alveoli. Further, it appears very striking that the connective tissue is diffusely infiltrated by mononuclear leukocytes. This round cell infiltration, which consists principally of small lymphocytes, showed the same striking characteristic finding in all our cases. Between these round cells we saw, in addition, a great number of plasma cells, the protoplasm and nuclei of which stained quite typically. The round cell infiltration chiefly involved the areas about a few larger vessels." (pp. 228–229)

Hashimoto's versus Riedel's struma. Hashimoto then compared Riedel's struma with struma lymphomatosa; in each there was no history of previous illness. Riedel's patients were in the third, and his, in the fifth decade of life. Hashimoto's 4 patients were women while Riedel's, with one exception (Kardua's patient), were males. In Hashimoto's struma, the thyroid had a tough consistency, but in Riedel's struma the gland was as hard as iron. In Hashimoto's struma, at operation it was difficult to free the thyroid capsule, but in Riedel's report, it was stated that complete extirpation was impossible.

"Round cell" infiltration was described in both conditions, according to the Japanese worker, as was proliferation of connective tissue and atrophy of alveoli. Riedel did not mention lymph follicle formation in his strumas since his attention apparently was fixed on the gross and microscopic feature of intense fibrosis. Hashimoto further emphasized that in both states, during the postoperative course, the remaining portion of the enlarged thyroid gradually receded.

Hashimoto concluded that there was extreme proliferation of the lymphatic elements, especially of lymph follicles, that the struma in advanced form had a tough consistency which could simulate a malignant tumor, that operation was followed by disappearance of the tumor, but that one should avoid extensive resection.

Interpretation of various writers. With the passage of time Riedel and Hashimoto were variously interpreted. Boyd,[2] for example, remarked that "areas of irregular cellular hyperplasia as well as the pale germ centers of the lymph follicles may persist" in Riedel's struma, although Riedel gave no similar description. As for Hashimoto's disease, Boyd stated that "fibrosis is also present but is not a marked feature," although the Japanese worker emphasized the striking fibrosis. Foot,[6] in his textbook, omitted any mention of the stroma in Hashimoto's disease.

Additional evidence that the criteria for the diagnosis of the two types of struma were not being observed was furnished by reports of cases in which the pathologist found it difficult to decide whether struma fibrosa or struma lymphomatosa was present or in which the two states were described as being simultaneously present. Means[17] described two cases (13796, 10824) in his book, "The Thyroid and Its Diseases". In the 1937 edition (p. 506) he doubted that the two types of struma were fundamentally different, but in the second edition (1948), he agreed (p. 483) that Riedel's and Hashimoto's strumas were separate entities. More recently, Merrington[18] reported a case of thyroiditis "with features of both Riedel's and Hashimoto's thyroiditis." The author called the areas with fibrosis, Riedel's disease, and considered those with "marked lymphocytic infiltration and degeneration [!] characteristic of Hashimoto's disease."

McGavack[16] added new features to both strumas. He stated that "signs of hyperthyroidism are relatively common, especially in the early states" of Riedel's disease. He also recorded another hitherto unmentioned factor: "In sharp contrast to Riedel's struma, significant alterations in the vascular wall [in Hashimoto's disease], other than those associated with coincidental arteriosclerosis, do not occur."

Lymphadenoid goiter. According to McGavack, Hashimoto's disease is the same as lymphadenoid goiter, an opinion shared by Hellwig[8] who described the microscopic picture as the same in both conditions. The latter, however, vitiated his comparison by noting that in none of his 4 cases was there any increase in connective tissue; Hashimoto had emphasized fibrosis as a cardinal sign. It seems reasonable, then, that "lymphadenoid goiter" perhaps ought to be reserved for those cases which have profound lymphocytic infiltration and lymphoid tissue formation, and atrophy of epithelium, but no fibrosis.

The role of thyroid function in lymphadenoid goiter is of interest. Hellwig wrote that the characteristic histologic changes of this type of goiter may be present in typical exophthalmic goiter, a "fact of particular interest [that] has been overlooked by most authors." Almost a fourth of his cases of exophthalmic goiter showed small islands of tissue alteration like those in lymphadenoid goiter. Beattie and Dickson[1] went further than Hellwig and called this condition a "secondary change in goitres." They wrote as follows:

> "Thus, in the latter stages of a primary hyperplastic toxic (primary exophthalmic) goitre, there may be degeneration and general atrophy of the epithelium as the result of exhaustion. In some cases, the degeneration and atrophy are focal, and around and obscuring the degenerating acini, collections of lymphocytes appear, sometimes even forming lymph nodes. The name 'lymphadenoid goitre' has been applied to this type of change which is best regarded as a regression than as a special type of goitre. Some examples of Riedel's struma may represent a final state of this regression" (pp. 830-831).

At this juncture it may be worthwhile to consider the status of lymphoid tissue in the thyroid gland. It is the experience of any morbid anatomist who has access to a large necropsy service to find a small proportion of glands with lymphoid tissue. Warthin[26] referred to a constitutional factor, the Graves constitution, underlying both toxic adenoma and exophthalmic goiter, and stated that most important and constant in the pathology of exophthalmic goiter was hyperplasia of the primitive lymph nodes of the thyroid. He asserted that there were microscopic differences between slightly and over-iodized thyroids, and that the latter, among other things, had lymphoid tissues with hyperplastic germ centers and lymphoid exhaustion. Warthin remarked that "Many of these over-iodized cases show a diffuse lymphoid infiltration, and a proliferation of the stroma and present the microscopic features of a so-called ligneous thyroiditis or Riedel's struma." Here again, microscopic features described by Hashimoto in struma lymphomatosa were classified with the Riedel label.

Hellwig's explanation of the mechanism of production of lymphadenoid goiter was not at variance with that of Beattie and Dickson. He regarded it as a disturbance of the normal thyroid cycle, with release greater than manufacture and storage. The loss of colloid, then, was a symptom of hypothyroidism. The lymph-

adenoid foci in almost 25 per cent of exophthalmic goiters, according to Hellwig, represented areas where the ability to synthesize intrafollicular iodine had been lost; most of the gland, however, had still retained that property. The cause of the disturbance was suggested as an excess of thyrotropic hormone.

There was no mention of fibrosis in Beattie and Dickson's account of lymphadenoid goiter, nor was fibrosis demonstrated in their photomicrograph of the condition. In short, lymphadenoid goiter is not the same as Hashimoto's struma, and, as Beattie and Dickson report and Hellwig suggests, probably represents a later or exhausted phase of hyperthyroidism or of the Graves constitution.

Vaux,[24] in 1938, described 38 cases of what she designated "lymphadenoid goitre" and employed the term to include cases of what Hellwig called by that name, as well as instances of thyroid disease which satisfy the criteria of Hashimoto's struma. Her attempt to identify some cases as those of Riedel's struma must be challenged; they did not fulfill the standards of that condition either in word or picture, but they could readily be recognized as examples of struma lymphomatosa. The same comment holds true for the report of Boyden *et al.*[3] of Riedel's struma in which both illustrations and textual descriptions were those of Hashimoto's disease. Is lymphadenoid goiter, then, a forerunner of struma lymphomatosa or struma fibrosa, and are the latter related to each other?

Current opinions. Despite Hashimoto's declaration of the many points of similarity between Riedel's struma and the struma described by him ("struma lymphomatosa," as employed by Hashimoto, and "lymphadenoid goiter" are not synonyms), at present the opinion is commonly held that there is no demonstrable relationship between the two conditions. Crile[4] for example, included them in a trilogy of thyroiditis, all considered unrelated to each other. The third of the group was that of subacute (pseudotuberculous or giant cell) thyroiditis.

Marshall *et al.*[13] had yet another classification of "chronic thyroiditis," unrelated to hypoplasia, adenomatous goiter, or to tumor. They employed three categories. The first category was subdivided into two types, both of which expressed reactions "that seem to be due obviously to infection," as also suggested by McClintock and Wright.[15] The first type was one of subacute inflammation in which neutrophilic leukocytes were identified, there was spillage of colloid, and foreign body giant cells were present. One subtype was Riedel's struma described as the terminal or fibrotic stage (of infection) in which there was little sign of inflammation, and foreign body giant cells were usually absent. Marshall's second category was Hashimoto's struma and the third, chronic nonspecific thyroiditis.

The *sex distribution* of struma fibrosa and struma lymphomatosa has been discussed. Hashimoto's 4 patients were women, and this feature has been frequently emphasized. For example, of the 78 cases of this disease collected by Marshall *et al.* only one case was seen in a male. Crile's[4] series of 14 patients consisted of 2 men and 12 women. McClintock and Wright, on the other hand, reported that Riedel's struma occurred with the same sex distribution as in other thyroid diseases. Crile, as well as Marshall, however, recorded a predominance of their cases in female patients. It appears, then, that Riedel's struma and Hashimoto's

disease do not differ in frequency in the sexes from that of thyroid disease generally.

Microscopically, in Riedel's disease McClintock and Wright[15] observed diffuse fibrosis of the affected portion of the thyroid with varying numbers of surviving acini. Where the acini were intact, they were grouped in clusters and small nests, and appeared to be compressed by the surrounding dense fibrous stroma. The cells of the remaining alveoli were not remarkable. Accumulations of lymphocytes, and occasionally of other leukocytes were found, but these were focal and appeared to be definitely inflammatory. Lymph nodules, so prominent in Hashimoto's struma, were not found. In 7 of 11 cases of Riedel's struma, Crile[4] found that "the inflammatory reaction appears to center about a degenerating adenoma." The fact that Riedel never gave a detailed microscopic report of the findings in his cases perhaps explains much of the confusion concerning the criteria for the diagnosis and understanding of the condition.

Hashimoto's disease is generally regarded as being originally bilateral and remaining so throughout the disease, but it is still disputed whether Riedel's struma is a unilateral affliction. In the series of McClintock and Wright,[15] 30 per cent were unilateral, while of the 11 cases recorded by Crile, 5 were initially bilateral and 6 unilateral. Of the latter, 2 later developed involvement of the other lobe. In summary, the thyroid is apparently diffusely affected in both types of struma, even though all areas are not necessarily affected to the same degree at the same time.

Evidence of hyperthyroidism in Riedel's struma is rare and equivocal. Crile[5] wrote that he had never encountered a single instance. Wahlberg[25] reported it present in the only case of that condition among the 2350 cases of thyroid disease in his personal practice. Nowhere in his monograph did Wahlberg establish his criteria of struma fibrosa and of struma lymphomatosa. In addition, the description of "granulocytes" suggested the presence of a primary inflammatory process. By the time the state of Riedel's disease is reached it is likely that the thyrotoxicosis has "burnt itself out".

In the cases reported by Marshall *et al.*,[13] hypothyroidism was present in 7 per cent of Riedel's struma before, and in 27 per cent after, operation. The basal metabolic rate of Crile's[4] patients ranged from minus 2 to plus 28 per cent (average, plus 2 per cent). McClintock and Wright[15] reported that 26 per cent of patients with Riedel's struma developed hypothyroidism postoperatively compared to 75 per cent of patients with Hashimoto's disease as recorded in other published reports.

As for *hyperthyroidism in struma lymphomatosa*, Wahlberg[25] reported that one of his 4 patients fell into that category. Clinical suspicion of thyrotoxicosis was confirmed by the patient's reaction to preoperative therapy. Two of the 9 patients of Boyden, Coller and Bugher[3] had clinical evidence of thyroid intoxication. In general, however, the basal metabolic rate has not been described as elevated to thyrotoxic levels in Hashimoto's struma. This was confirmed by Vaux;[24] of 10 patients in this category, 7 had hypothyroidism and none, evidence of toxicity.

REPORT OF CASES

Case 1

A 20 year old white woman (45–6854) underwent partial thyroidectomy in October, 1945. For three years she had had "choking spells" and progressive enlargement of the thyroid gland. The basal metabolic rate was plus 30 per cent, and an iodine preparation was given for about a month before operation.

Each of the two similar specimens (45-1802) from the lateral lobes measured 3.5 x 2 x 4 cm. Normal pink tissue was largely replaced by gray lobulated tissue, and it was noted that the picture suggested Hashimoto's disease. Microscopically, the normal architecture was no longer discernible, and in its place was a basic structure of lymphoid tissue with well-formed follicles having large germinal centers (Fig. 1, A). Throughout the lymphoid tissue small foci of atrophic small acini were seen; some of the latter were without lumens (Fig. 1, B), and others contained a small amount of thin colloid. There were also areas with relatively normal appearing acini which were separated by dense connective tissue or by proliferating connective tissue. The diagnosis made at the time was struma lymphomatosa (Hashimoto).

Case 2

A 30 year old overweight white woman (45–7057) had a subtotal removal of an enlarged thyroid in November, 1945. She had had profound psychic disturbance including fear of fainting in crowded places, severe perspiration without exertion, and tiredness. The basal metabolic rate before operation was plus 25 per cent and iodine therapy was employed. Five months after thyroid resection, the rate was plus 10 per cent.

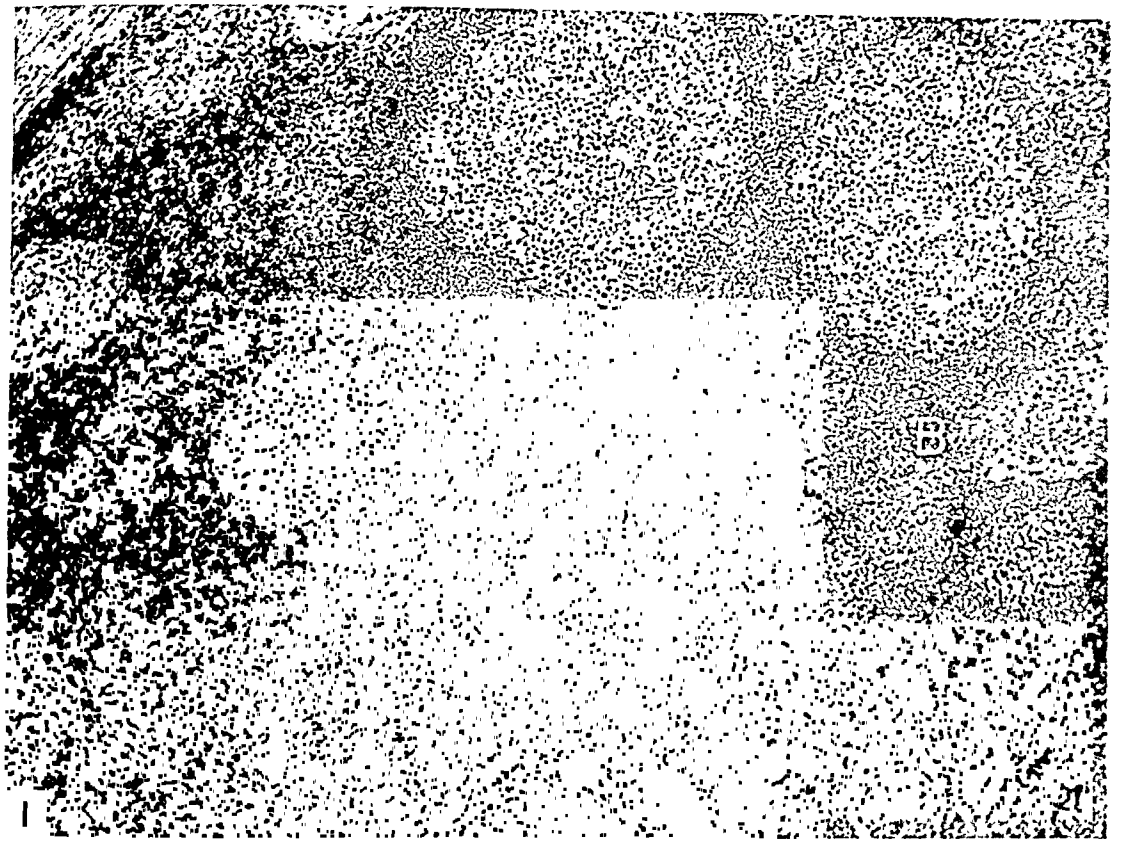
The resected lateral lobes (45-1858) were each about 3 x 3 x 1 cm. and were described by the surgeon as "enlarged and fibrotic." On section, the indefinitely nodular tissues were gray to tan to deep pink. In one lobe there was a circumscribed tan nodule, 1.5 cm. in maximum diameter. All microscopic sections indicated that the normal thyroid structure was disturbed; it was largely obliterated by large areas of lymphoid tissue with genuine follicles (Fig. 2, A). Where the lymphoid tissue was less pronounced, there was a fine reticulum with lymphoid cells in the interstitial stroma. As a result, the thyroid acini (Fig. 2, B) were separated from each other but were generally small and without lumens. Where lumens were present, they were filled with dense nonvacuolated colloid. Fibrosis was prominent in some areas. Here, too, the immediate diagnosis was struma lymphomatosa (Hashimoto).

Case 3

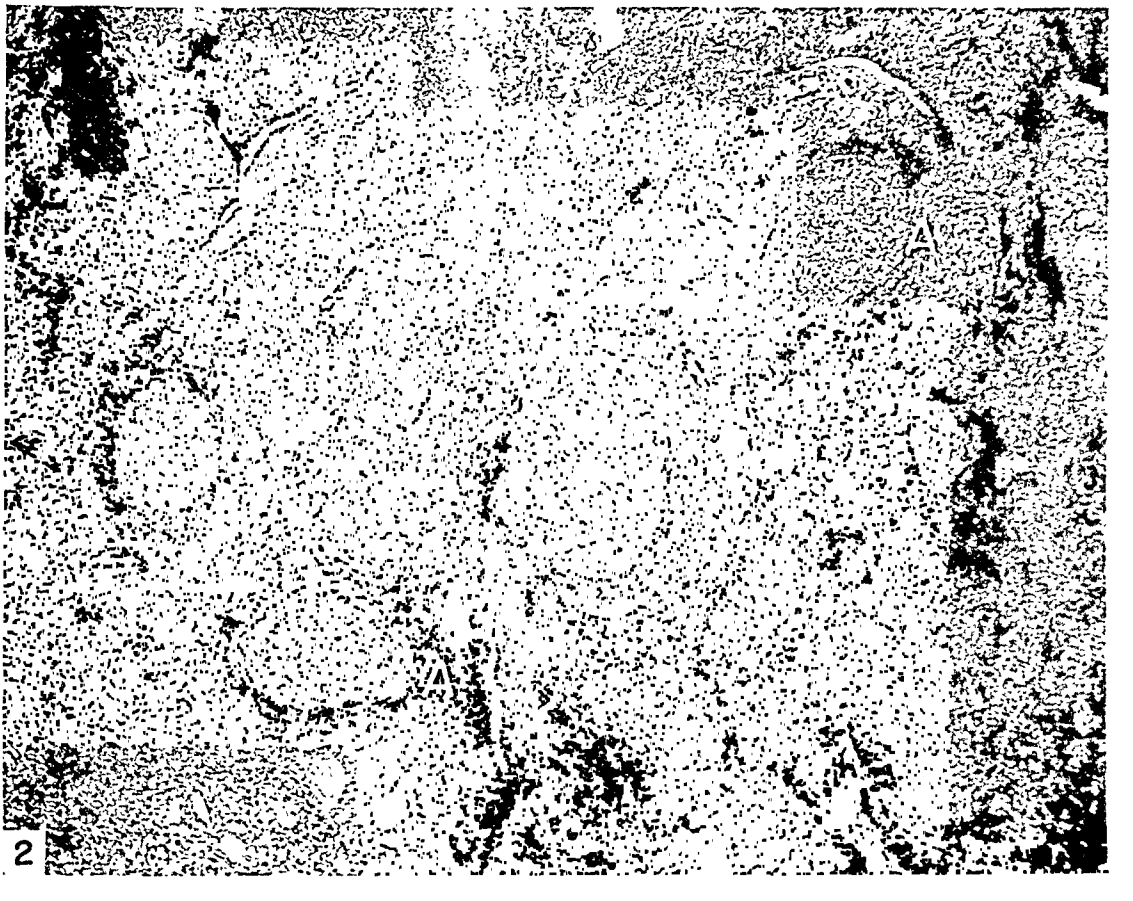
Clinical history. A 27 year old white man, who had not felt well for the preceding six months, noticed rapid enlargement of the neck in December, 1945. A weight loss of 55 pounds

FIG. 1. Case 1. Lymphadenoid goiter, showing marked lymphoid tissue hyperplasia (A). The atrophic thyroid acini (B) are masked by lymphoid tissue. Hematoxylin and eosin. × 110.

FIG. 2. Case 2. Lymphadenoid goiter, showing marked lymphoid tissue hyperplasia (A). The degree of thyroid acinar atrophy (B) is less marked than in Figure 1. Hematoxylin and eosin. × 66.



1



2

FIGS. 1–2

was attributed to a diabetic state of nine years' standing. The swelling of the neck persisted. He developed a sensation of choking and shortness of breath and consulted a physician in January, 1946. His mother had died following a thyroid operation and the maternal grandmother had had diabetes. The thyroid was diffusely enlarged, firm and smooth. There was marked tremor and exophthalmos and the basal metabolic rate was plus 47 per cent. Despite the use of phenobarbital and thiouracil, there was no improvement and a month later the metabolic rate had climbed to plus 71 per cent. Lugol's solution was now prescribed, the nervousness decreased and he gained 40 pounds in weight. On July 1, 1946, the patient was admitted (46–4281) to the hospital. The basal metabolic rate just before admission was plus 50 per cent; the blood sugar level was not at normal levels although a total dose of about 100 units of insulin was administered daily. At operation, July 2, the surgeon was unable to remove all of the very firm and smooth right lateral lobe because of its firm adherence to the surrounding structures.

Pathologic anatomy. The operative specimen (46-1146) measured 7 x 5.5 x 3 cm. and weighed 57 Gm. It was composed of closely packed indefinitely lobulated pale gray-tan tissue. Microscopically, there was a distinct lobular arrangement of thyroid substance with septa of varying thickness and density (Fig. 3). The first striking feature was the absence of lumens in most acini (Fig. 3, A). Only a few distinct lumens were present and these were generally empty. The acini with the smallest lumens had papillary intrusions. The single layer of alveolar epithelium was cuboidal to low columnar and indefinitely finely granular. The absence of lumens was more apparent than real, because the very finely reticulated colloid in the lumens blended with the ctyoplasm of the adjacent cells. The alveoli were regular as were their cells, and mitotic figures were not found. It was the increase of interlobular and interstitial connective tissue which was responsible for the firm consistency of the specimen. The connective tissue in some places was dense, acellular and collagenous (Fig. 4, A); in other areas, it was less mature, being more cellular, looser and more vascular. The process of fibrosis could be followed from relatively early proliferation to the end-stage of dense fibrous tissue. Another prominent feature was the finding of large areas of true lymphoid tissue (Fig. 3, B) with follicles and germinal centers (Fig. 4, C). Such foci were found largely within the lobules, but they were also seen in the septa.

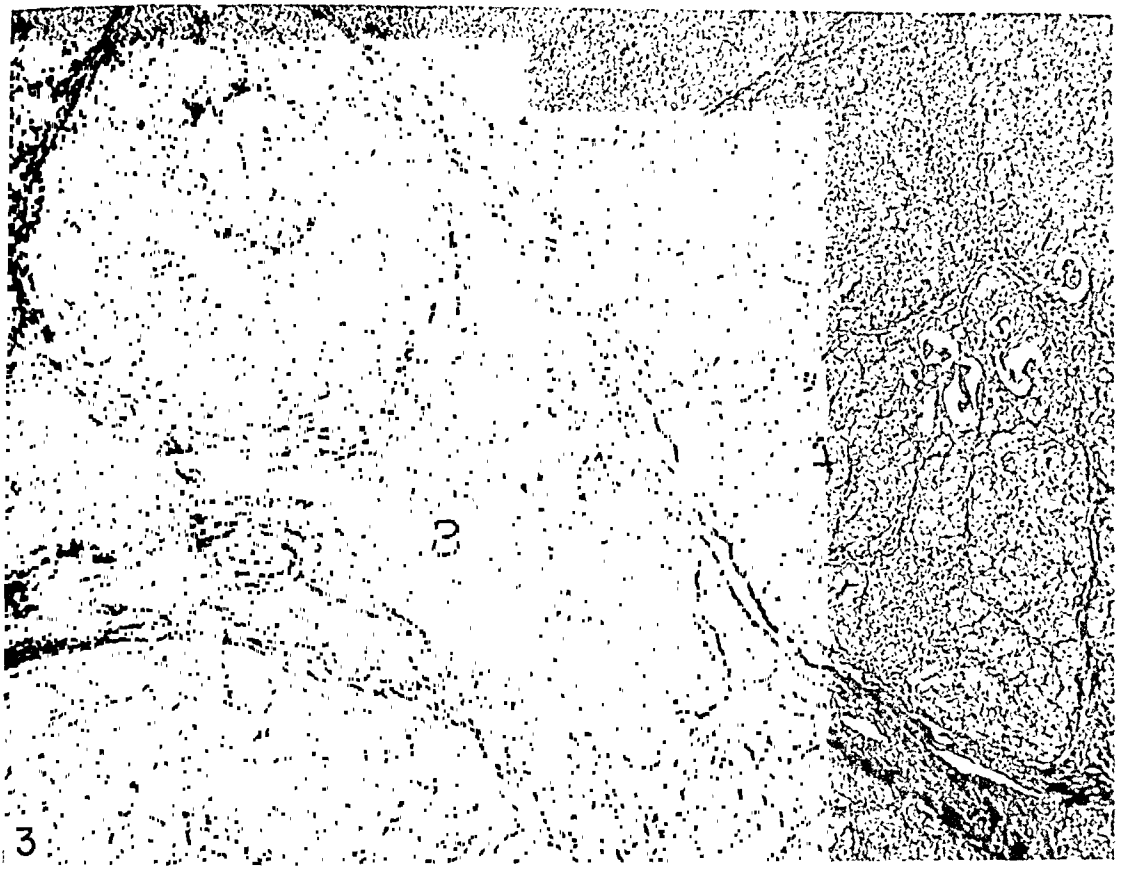
The diagnosis was Graves' disease of the less common type in which the acini were not dilated but merely increased in number. As in the usual type, there was thinning and disappearance of colloid and marked hyperplasia of the lymphoid apparatus. Nowhere was there evidence of neoplasia.

Postoperative course. Following operation, the patient continued to receive thiouracil and phenobarbital and improvement was progressive. The basal metabolic rate in November 1946 was minus 5 per cent. In December, he was readmitted to the hospital for three days to control his diabetes, and upon discharge was advised to continue to take insulin and thiouracil. Dyspnea and a sense of choking were recorded as still present.

In March, 1947, the patient again entered the hospital (47–1946) for the removal of the

FIG. 3. Case 3 (1st operation). Lymphadenoid goiter; hyperthyroidism. Areas of epithelial hyperplasia (A) separated by large fields of lymphoid tissue (B). Hematoxylin and eosin. × 85.

FIG. 4. Case 3 (1st operation). Lymphadenoid goiter; hyperthyroidism. Obvious fibrosis (A) disturbs the normal lobular pattern of acini (B). Lymphoid tissue (C) is prominent. Hematoxylin and eosin. × 60.





FIGS. 3-4

other (left) lateral lobe of the thyroid gland. Difficulty in breathing and the choking sensation were more severe, but there was no clinical evidence of hyperthyroidism. He demanded operation because he regarded the gland as physically disfiguring. The gland was smooth, very hard and peripherally firmly fixed to the surrounding structures.

Operation. At operation, the surgeon was again amazed at the hardness of the gland. It seemed to be even more adherent to the surrounding structures than had been the lobe which was partially removed in July 1946. Again the physician was unable to resect the entire lobe and had to be satisfied with removing a portion of it. He feared he was dealing with carcinoma of the thyroid.

Pathologic anatomy. The single specimen (47-0444) measured 6.5 x 4 x 2.4 cm. and weighed 36 Gm. It was partly encapsulated and partly lobulated, gray and firm. On section, there were small fields of grayish tan tissue surrounded by thicker and thinner strands of obvious scar tissue. Several sections presented a similar microscopic picture. Only in a few places was one able to identify structures definitely resembling thyroid acini (Fig. 5, A). The gland was largely replaced by interlacing bundles and strands of dense collagenous tissue, a moderate amount of cellular connective tissue, and numerous islands of lymphoid tissue having large germinal centers (Fig. 5, B). The surviving thyroid acini were small and lined by one to three layers of cuboidal, polyhedral cells with indistinct boundaries. There was no colloid in the lumens and, in many places, the latter were almost completely obliterated by the compression of the acini by the dense connective tissue. In many places one was not certain whether the cells were lymphoid or acinar cells. No mitotic figures were noted in the epithelial cells and only a few in the lymphoid follicles.

In only one microscopic section was there evidence of epithelial hyperplasia. In a small area there were several foci of closely placed round to oval acini in very scanty stroma. The alveoli were formed of a single layer of tall cuboidal to tall columnar vesiculated epithelial cells with single regular nuclei which virtually showed no mitosis. The lumens were either absent or extremely small. Vascularity was not a prominent feature of the specimen. Foci of calcium were found in the walls of a few vessels. The diagnosis submitted was fibrosis of thyroid (Riedel's type), and residual focal hypertrophy of thyroid.

Dr. Hellwig[9] kindly furnished microscopic slides of a case in which the changes resembled those of the two specimens of Case 3. A 61 year old obese white woman had progressive painless and otherwise symptomless swelling of the neck for a year. A few weeks before operation, choking sensation and mild toxic symptoms appeared. The basal metabolic rate a week before operation was plus 26 per cent and, six days later, after iodine therapy, plus 19 per cent. The blood cholesterol was 88.2 mg. The thyroid gland, which was firm and diffusely enlarged, extended behind and was firmly adherent to the trachea. There was an uneventful recovery. The patient was discharged on the tenth day.

The operative specimen weighed 105 Gm.; the left lobe measured 7 x 6 x 3 cm., the right lobe, 9 x 6 x 3 cm., and the isthmus was 2 cm. wide. The cut surface, which was free of nodules, was pale gray. The tissues were very hard, especially near the surface, and had a gritty character when cut. Microscopically, most areas resembled those of the first specimen of Case 3, but there were foci of dense fibrosis and parenchymal atrophy like those in the second specimen.

COMMENT

It was clear, after reviewing the papers of Riedel and Hashimoto, as well as of other authors, that the original diagnosis in our 3 cases would have to be revised. Cases 1 and 2 (both patients were women) now are regarded as instances of lymphadenoid goiter, as the term is used by Hellwig.[8] The relatively slight degree of fibrosis might be interpreted as of little significance or else as pointing

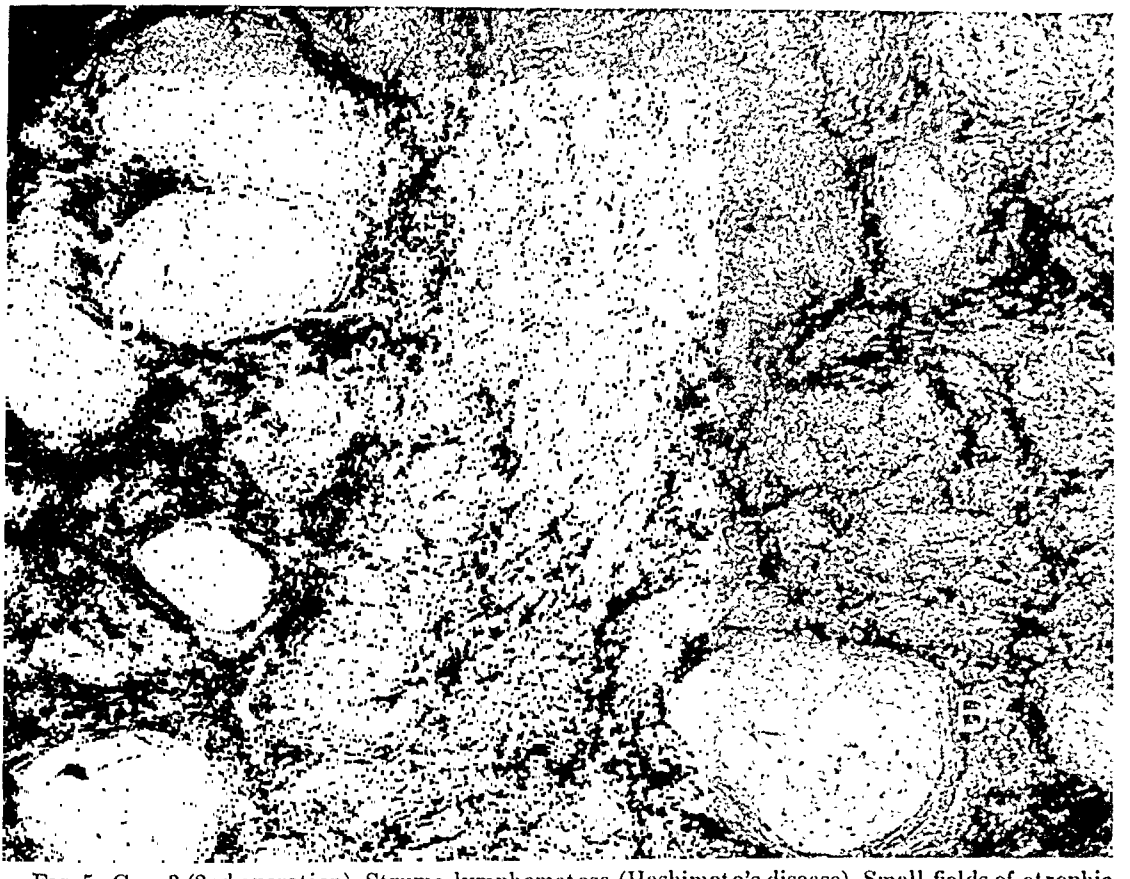


FIG. 5. Case 3 (2nd operation). Struma lymphomatosa (Hashimoto's disease). Small fields of atrophic thyroid acini (A) survive despite the intense fibrosis (black areas). Lymphoid hyperplasia with follicle formation (B) is prominent. Trichrome stain (Masson-Goldner-Foot). × 31.

to future greater fibrosis, had the operations not been performed. In the third case, that of the young man, without knowledge of the previous history, the diagnosis must be Hashimoto's struma. At the time the second specimen was obtained, the basal metabolic rate was well within normal limits. The thyroid gland was firmly adherent to the adjacent tissue, perhaps more firmly than originally described by Hashimoto. Grossly and microscopically, the second specimen was that of struma lyphomatosa since it showed the following features: richness of lymphoid tissue, marked fibrosis and severe atrophy of epithelial elements.

What about the first specimen in Case 3, obtained during a period of marked thyrotoxicosis? The general paucity of profound epithelial atrophy and the less well pronounced fibrosis is the anatomic evidence, and the emphatically elevated basal metabolic rate, the physiologic evidence against Hashimoto's struma and for lymphadenoid goiter. Here, in a single patient, are gathered the links uniting the latter two states.

Were it not for the presence of lymphoid tissue, the diagnosis of the second specimen of Case 3 logically would be Riedel's struma. It is assumed, of course, that Riedel[20–22] did not mention lymphoid tissue in his brief microscopic description of the lesion that bears his name because lymphoid tissue was not present. In short, the presence or absence of lymphoid tissue, as Hashimoto indicated, is probably the basic factor in distinguishing struma lymphomatosa from Riedel's disease.

It may be suggested, then, that Hashimoto's struma represents the end-stage in the involutionary history of the Graves' constitution, with or without thyrotoxicosis, with lymphadenoid goiter as an interim phase. It must be emphasized that the evolution from the earliest to the latest stages can stop at any level, either temporarily or permanently. The views of Vaux[24] thus are in part reiterated and those of Joll[10] contradicted. Boyden, Coller and Bugher[3] expressed similar ideas if, for "Riedel's struma" as expressed in their title, there is substituted "Hashimoto's disease" of which their cases were representative. They wrote that " . . . the peculiar pathological changes . . . are largely the result of prolonged iodine ingestion in individuals of the Graves constitution type." They assumed that their patients, living in the "goiter belt," had taken iodine although such a history was obtained only in a minority. They did not, however, regard iodine as the sole etiologic agent. Iodine perhaps exaggerates other influences as yet to be identified, but apparently is not essential to the production of the thyroid changes described in the present paper.

Shaw and Smith,[23] almost 25 years ago, described 6 cases of "Riedel's chronic thyroiditis," including the condition described by Hashimoto[7] in 1912 as "struma lymphomatosa". They regarded the latter as the early stage of Riedel's disease. Their Cases 1 and 2, described as being without appreciable fibrosis, were likened to those reported by Hashimoto, but from the description and photomicrographs, both instances might better be diagnosed as lymphadenoid goiter. Their Case 4, twice operated upon, satisfies the criteria of advanced struma lymphomatosa, and Cases 3 and 6, of Riedel's struma. The authors explained the absence of myxedema by the survival of enough normal thyroid epithelial tissue. They concluded that struma fibrosa is a chronic granulomatous inflammatory disease.

That Hashimoto's and Riedel's strumas have been regarded as inflammatory states has already been discussed. The recent report of Patterson and Starkey,[19] devoted to these conditions, included them in the category of chronic thyroiditis. The authors rejected the view that the two states were related; clinically, however, there was close approximation. They failed to indicate the cause of the inflammation. In discussing the paper, Marshall[14] reaffirmed that Riedel's struma was the end-stage of infection, and that struma lymphomatosa fell into the

group of "nonspecific" chronic thyroiditis. He also stated that the reaction may be due to irritation or to trauma, neither of which was defined. Patterson and Starkey did not describe the relationship of the Graves constitution to the struma, although there was a brief reference that in the early stages of Hashimoto's disease there may be "a transient hyperthyroidism"; that statement was not amplified.

Other writers also agree that Riedel's struma is a terminal phase, equivalent to struma lymphomatosa, in which adenomatous goiter is present. Karsner[12] wrote that in Riedel's disease "adenomatous foci of hyperplasia may be found." In 7 of 11 cases of this condition Crile[4] uncovered degenerating adenomas apparently serving as nuclei for the fibrotic reaction and added that "this may be the clue to its etiology." Marschall *et al.*[13] also made the observation that "Examples of such change [reaction to injury or irradiation] are seen in the pathologic states of hyperplasia, adenomatous goiter and tumor. In hyperplasia fibrosis . . . is frequent." Reasonable objections to this thesis have been presented (Vaux)[21] that the presence of adenomata is coincidental and unrelated to the primary pathologic process, a position with which the present authors tend to agree.

While Vaux's claim is not unlikely, that Riedel's struma is a late phase of Hashimoto's disease, it could not be substantiated from the textual descriptions or from the photomicrographs of her report. What she identified as the late state and termed Riedel's struma must be regarded as struma lymphomatosa because of the presence of a rich supply of lymphoid tissue. This, however, does not gainsay the reasonableness of her premise that struma fibrosa is *the* end stage. The clinical alterations of the thyroid activity represented anatomically by Hashimoto's and Riedel's strumas make it not unlikely that the latter is the successor to the former. The much greater adherence of the struma fibrosa to adjacent structures also points to greater fibrosis, and in the intense fibrotic process the lymphoid tissue, still surviving in the "Hashimoto" phase, may succumb, thus producing the "Riedel" or end-stage.

SUMMARY

1. Three cases of thyroid disease are reported, in the third of which two operations were performed. Similar findings were encountered in the first two cases and at the first operation of the third case; these were considered compatible with a diagnosis of lymphadenoid goiter. The second specimen of the last case was diagnosed as struma lymphomatosa (Hashimoto's disease).

2. In the involutional history of goiters of persons with the Graves' constitution a sequence beginning with lymphoid hyperplasia, may be identified as follows: lymphadenoid goiter, struma lymphomatosa (Hashimoto), and struma fibrosa (Riedel). The involution may stop at any level, either temporarily or permanently. Factors influencing the thyroid state may influence the rate and severity of the progression.

Acknowledgments. Thanks are due to Dr. L. K. Gould and Dr. E. H. Kruse for furnishing clinical data, and to Mrs. Grace Brooks for her technical assistance.

REFERENCES

1. Beattie, J. M., Dickson, W. E. C., Drennan, A. M., and Oliver, J. O.: Textbook of Pathology, General and Special. Ed. 4. London: William Heinemann, pp. 823, 830–831, 1943.
2. Boyd, W.: Surgical Pathology. Ed. 6. Philadelphia: W. B. Saunders Company, p. 201, 1947.
3. Boyden, A. M., Coller, F. A., and Bugher, J. C.: Riedel's struma. West. J. Surg., **43:** 547–563, 1935.
4. Crile, G., Jr.: Thyroiditis. Ann. Surg., **127:** 640–654, 1948.
5. Crile, G., Jr.: Personal communication to senior author.
6. Foot, N. C.: Pathology in Surgery. Philadelphia: J. B. Lippincott Company, p. 325, 1945.
7. Hashimoto, H.: Zur Kenntnis der lymphomatösen Veränderungen der Schilddrüse (Struma lymphomatosa). Arch. f. klin. Chir., **97:** 219–248, 1912.
8. Hellwig, C. A. Lymphadenoid goiter. Arch. Path., **25:** 839–849, 1938.
9. Hellwig, C. A.: Personal communication to senior author.
10. Joll, C. A.: Pathology, diagnosis, and treatment of Hashimoto's disease (struma lymphomatosa). Brit. J. Surg., **27:** 351–389, 1939.
11. Kardua: Discussion in paper of Riedel.[20]
12. Karsner, H. T.: Human Pathology. Ed. 6. Philadelphia: J. B. Lippincott Company, p. 691, 1942.
13. Marshall, S. G., Meissner, W. A., and Smith, D. D.: Chronic thyroiditis. New England J. Med., **238:** 758–766, 1948.
14. Marshall, S. F.: Discussion to Patterson and Starkey.[19]
15. McClintock, J. C., and Wright, A. W.: Riedel's struma and struma lymphomatosa (Hashimoto); a comparative study. Ann. Surg., **106:** 11–32, 1937.
16. McGavack, T. H.: Thyroid gland. In: Practice of Medicine, by F. Tice. Hagerstown, Md.: W. F. Prior Company, Vol. 8, pp. 222, 224–225, 1931.
17. Means, J. H.: The Thyroid and Its Diseases. Ed. 1. Philadelphia: J. B. Lippincott Company, 602 pp., 1937; Ed. 2, pp. 476–483, 1948.
18. Merrington, W. R.: Chronic thyroiditis; a case showing features of both Riedel's and Hashimoto's thyroiditis. Brit. J. Surg., **35:** 423–426, 1943.
19. Patterson, H., and Starkey, G.: Clinical aspects of chronic thyroiditis. Ann. Surg., **128:** 756–769, 1948.
20. Riedel, B.: Die chronische, zur Bildung eisenharter tumorenführende Entzündung der Schilddrüse. Verhandl. d. deutsch. Gesellsch. f. Chir., **25:** 101–105, 1896.
21. Riedel, B.: Vorstellung eines Kranken mit chronischer Strumitis. Verhandl. d. deutsch. Gesellsch. f. Chir., **26:** 127–129, 1897.
22. Riedel, B.: Ueber Verlauf and Ausgang von Strumitis chronica. München. med. Wchnschr., **57:** 1946–1947, 1910.
23. Shaw, A. F. B., and Smith, R. P.: Riedel's chronic thyroiditis: with a report of six cases and a contribution to the pathology. Brit. J. Surg., **13:** 93–108, 1925.
24. Vaux, D. M.: Lymphadenoid goitre; study of thirty-eight cases. J. Path. and Bact., **46:** 441–446, 1938.
25. Wahlberg, J.: Studien über die Schilddrüsenkrankheiten in Finnland. Acta med. Scandinav., Suppl. 94, 298 pp., 1938.
26. Warthin, A. S.: Pathology of goiter. Proc. Inter-state Postgrad. Med. Assembly N. America. Milwaukee: Edwin Heines, Jr., p. 383–385, 1929.

CYTOLOGIC DIAGNOSIS OF BRONCHOGENIC CARCINOMA*

LEWIS B. WOOLNER, M.D., AND JOHN R. McDONALD, M.D.

From the Section on Surgical Pathology, Mayo Clinic, Rochester, Minnesota

Diagnosis of bronchogenic carcinoma by the smear technic has been the subject of considerable investigation since the original work of Dudgeon and Wrigley[1] in 1935. Preliminary studies by various authors indicate that carcinoma cells may be found in sputum or bronchial secretions in 60 to 80 per cent of the cases of cancer of the lung.

Cytologic examination of sputum and bronchial secretions has been carried out in the authors' laboratory for almost three years. During this period, sputum or bronchial secretions of approximately 3000 patients have been examined and a cytologic diagnosis of carcinoma has been made in 320 cases. Our experience falls into two stages. The first 150 positive diagnoses given on the basis of smears represent a period of study during which familiarity with cytologic methods was gradually acquired. An analysis of this first group of 150 cases in which the results were positive has been published by us. The second group of 150 positive cytologic diagnoses represents a period of evaluation of the method as a routine diagnostic procedure and forms the subject of this paper. During this period, cytologic studies have been made on the majority of patients with roentgenologic or other evidence of thoracic disease of any type. A comparison of the two series of cases is provided.

In order to determine the actual incidence of cases missed by routine cytologic studies (false negatives) it was decided to examine the case records of all patients on whom cytologic examination gave negative results during the period covering the last 100 cases in which positive results were obtained. Sputum or bronchial secretions of 488 patients were examined during this time and reported negative for carcinoma cells. The details of this cytologic study of 588 patients are reported below.

TECHNIC

The technic employed has been outlined in a previous paper by us.[3] Briefly, 5 smears prepared from each specimen are fixed, while wet, in alcohol-ether mixture according to the method of Papanicolaou.[2] As a routine procedure we have used Harris' hematoxylin with dilute eosin as a counterstain, and we have found this method of staining to be entirely satisfactory. This procedure gives adequate nuclear detail and has the added advantage of simplicity and familiarity to pathologists.

CRITERIA FOR CYTOLOGIC DIAGNOSIS OF CARCINOMA

The characteristics of exfoliated cancer cells as seen in sputum or bronchial secretions have been outlined by various authors. Variations in the appearance

* Received for publication, March 3, 1949.

of the cells, according to the histologic type of tumor in the bronchus, has been outlined by us previously[4, 5] and will not be repeated in this paper. In general, carcinoma cells can be distinguished from normal cells by their large size, variation in size and shape of the cells and of the nuclei, increase in the nuclear cytoplasmic ratio, hyperchromatism of the nuclei, and presence of coarse chromatin and prominent nucleoli.

In the majority of cases small clumps of carcinoma cells are present in the smear, and it is on such cell groups that the diagnosis is most readily based (Fig. 1*a*). It should be pointed out, however, that a diagnosis of carcinoma is just as readily made on scattered, isolated, single cancer cells (Fig. 1*b*). It is obviously unsafe to place too much reliance on one or two cells no matter how atypical, but in practice if one such cell is found, continued search will reveal others. In our routine work, a cytologic diagnosis of cancer is not infrequently made on a total of 10 or 15 cells scattered throughout the 5 slides. If fewer than this number are present, additional specimens are examined until sufficient evidence for a positive diagnosis has been accumulated.

Small cell ("oat-cell") bronchogenic carcinomas are the most difficult to diagnose from smears (Fig. 1*c*). The cells are of small size, sometimes only slightly larger than lymphocytes, and great care must be taken to avoid false-positive results in this group. Variation in size of the nuclei is probably the most important single feature in distinguishing these cells from normal.

ACCURACY OF DIAGNOSIS BY CYTOLOGIC EXAMINATION OF SPUTUM AND BRONCHIAL SECRETIONS

False-Positive Diagnoses

In the second series of 150 patients with positive cytologic evidence of carcinoma, the final diagnosis of dismissal from the clinic is of interest. In 89 cases a diagnosis of bronchogenic carcinoma was established by tissue section. In 55 cases tissue was not obtained and the diagnosis of bronchogenic carcinoma was based on clinical and roentgenologic evidence. This was supported by such secondary evidence as metastasis to bone or lymph nodes, fixed vocal cord, pleural effusion, Horner's syndrome, superior vena caval obstruction or the gross appearance at bronchoscopic examination. In all these cases the lesions were considered inoperable on surgical consultation. In 4 cases a known carcinoma was present elsewhere, namely, in the breast, rectum, prostate and uterus, so that the pulmonary lesion was presumably metastatic. In one case, a carcinoma of the hypopharynx was found, representing the only case in this series in which the malignant process was outside the lower part of the respiratory tract.

In one proved case, a false-positive result was obtained on the basis of smears. The cytologic diagnosis was small-cell carcinoma. A right pneumonectomy was done and pathologic examination revealed only chronic pneumonitis. On review of the smears in this case, after further experience with small-cell carcinoma, we feel that the atypical cells responsible for the error represented closely packed collections of large lymphocytes.

The low incidence of false-positive results represents an improvement over that in the earlier series of 150 cases reported by us. In this earlier series, 3 false-positive results were given on the basis of smears, and in 2 other cases the final diagnosis concerning the inflammatory or neoplastic nature of the lesion was not established.

The value of the method as an adjunct to bronchoscopic biopsy is indicated by the relatively low rate of positive results on bronchoscopic biopsy in both series (Table 1). Many lesions in the upper lobes and periphery are out of reach of the bronchoscope, and in such cases cytologic studies provide the only means of making a microscopic diagnosis of cancer.

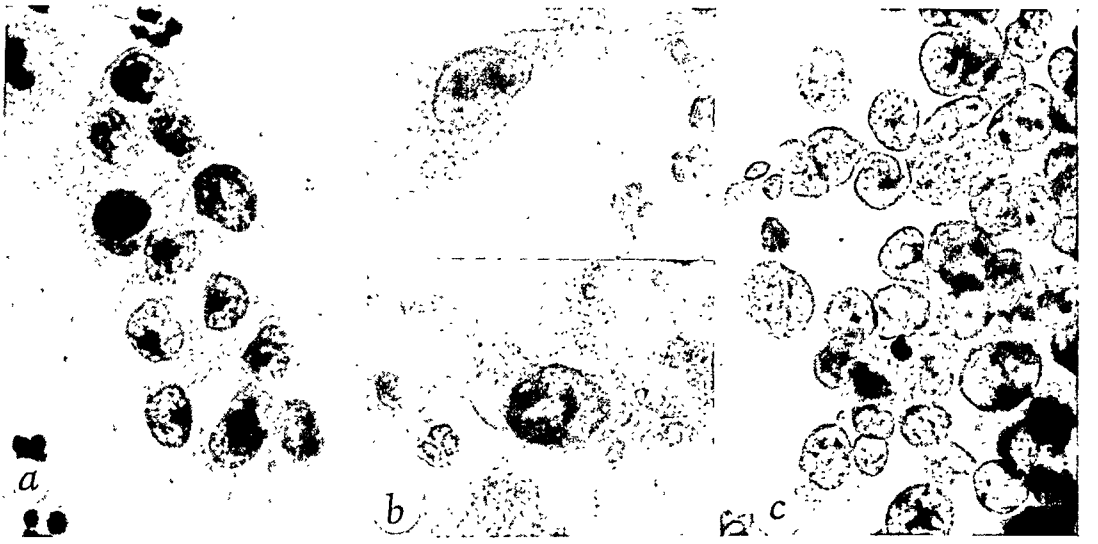


FIG. 1. Carcinoma cells in sputum (hematoxylin and eosin, × 750). a. Clump of carcinoma cells. b. Isolated cells found in different parts of the smear. Numerous other single carcinoma cells were present in this case. c. Clump of cells in the sputum from a patient with small-cell bronchogenic carcinoma. Note absence of cytoplasm and variation in size of nuclei. The cells in this case are somewhat larger than in the usual "oat-cell" carcinoma.

Our results indicate that use of the smear technic will reduce materially the number of preoperative diagnoses of "indeterminate pulmonary lesion". The number of cases in which exploration was carried out and the microscopic basis for the diagnosis are shown in Table 1. In both series in approximately 40 per cent of patients undergoing surgical exploration, smears presented the only preoperative microscopic evidence of cancer. Similar findings were noted in the cases in which pneumonectomy or lobectomy was done. Resection was done in 9 cases of the first series and in 14 cases of the second series on the basis of positive cytologic findings in sputum or bronchial secretions.

False-Negative Results

In order to determine the incidence of false-negative results in routine cytologic studies on patients with suspected pulmonary disease, an arbitrary period

covering our last 100 patients with positive results was selected. The records on all patients with negative results on cytologic study during this period were examined and the dismissal diagnosis on each patient noted. The patients on whom cytologic studies were carried out during this period were grouped as follows: patients with positive cytologic evidence, all of whom were believed to

TABLE 1

CARCINOMA CELLS IN SPUTUM OR BRONCHIAL SECRETIONS: VALUE OF CYTOLOGIC EXAMINATION AS AN ADJUNCT TO BIOPSY IN TWO CONSECUTIVE SERIES OF 150 CASES, EACH IN WHICH THE RESULTS WERE POSITIVE

	SERIES 1, CASES		SERIES 2, CASES	
Bronchoscopic examination	104		103	
Positive results in biopsy	66	(63%)	55	(53%)
Surgical exploration	58*		52†	
On basis of biopsy	31		31	
On basis of smear	27		21	
Pneumonectomy or lobectomy	24		32	
On basis of biopsy	15		18	
On basis of smear	9		14	

* Includes 2 false-positive results.

† Includes 1 false-positive result.

TABLE 2

FINAL DIAGNOSES IN 488 PATIENTS IN WHOM THE RESULTS OF CYTOLOGIC EXAMINATION OF SPUTUM FOR CARCINOMA CELLS WERE NEGATIVE

DIAGNOSES		NO. OF PATIENTS
Bronchogenic carcinoma proved by tissue section		21
Bronchogenic carconoma diagnosed primarily on the basis of clinical and roentgenologic evidence		18
Indeterminate, probable bronchogenic carcinoma		8
Total cases of proved or suspected bronchogenic carcinoma	47	
Indeterminate, probably non-neoplastic		39
Possible metastatic carcinoma of lung		30
Adenoma of bronchus		5
Non-neoplastic pulmonary disease		367
Total patients		488

have bronchogenic carcinoma, 100; patients with negative cytologic evidence, 488; and total patients examined, 588.

Of the 488 patients with negative results on cytologic study, sputum was examined in 404 and bronchial secretions in 199. Both sputum and secretions were examined in 116. The number of sputum specimens submitted ranged from 1 to 8, with an average of 1.78 per patient. One specimen of bronchial secretion was examined on each of 190 patients who underwent bronchoscopic examination and 2 specimens were examined on each of the remaining 9 patients. The final

diagnoses on the 488 patients, determined either clinically or at operation, are listed in Table 2. Forty-seven cases of proved or strongly suspected bronchogenic carcinoma were missed on routine cytologic examination. In 7 of these cases a single specimen of bronchial secretion was examined; in 21 cases sputum examination alone was carried out on an average of 2 specimens per patient, and in 19 cases both sputum and bronchial secretions were examined, an average of 2.3 specimens of sputum and one specimen of bronchial secretion being examined from each patient.

Calculated on the basis of the total number of 147 patients with bronchogenic carcinoma during this period, results of routine cytologic examination were positive in 100, representing positive findings in 68 per cent.

SUMMARY

A diagnosis of carcinoma based on cytologic examination of sputum or bronchial secretions has been made on 300 patients. This total has been divided into two groups, the first 150 cases representing a period of investigation with the method and the second 150 cases representing a period of evaluation of the method as a routine procedure in the diagnosis of thoracic diseases. Proved false-positive results in 3 cases in the first series and in one in the second series were reported. In a group of 588 patients with miscellaneous diseases of the thorax, on whom a routine cytologic examination of sputum or bronchial secretions was carried out, bronchogenic carcinoma was proved or suspected in 147. In 100 patients, or in 68 per cent of the total, the lesions were detected by a cytologic examination of sputum or bronchial secretions.

REFERENCES

1. Dudgeon, L. S., and Wrigley, C. H.: On the demonstration of particles of malignant growth in the sputum by means of the wet-film method. J. Laryng. and Otol., **50**: 752–762, 1935.
2. Papanicolaou, G. N.: A new procedure for staining vaginal smears. Science, **95**: 438–439, 1942.
3. Woolner, L. B., and McDonald, J. R.: Bronchogenic carcinoma; diagnosis by microscopic examination of sputum and bronchial secretions; preliminary report. Proc. Staff Meet., Mayo Clin., **22**: 369–381, 1947.
4. Woolner, L. B., and McDonald, J. R.: Carcinoma cells in sputum and bronchial secretions; a study of 150 consecutive cases in which results were positive. Surg., Gynec. and Obst., **88**: 273–290, 1949.
5. Woolner, L. B., and McDonald, J. R.: Diagnosis of carcinoma of the lung; the value of cytologic study of sputum and bronchial secretions. J. A. M. A., **139**: 497-502, 1949.

PROGRESSION FROM "PRECANCER" STAGE TO EARLY CARCINOMA OF CERVIX WITHIN ONE YEAR

Combined Cytologic and Histologic Study with Report of a Case*

J. ERNEST AYRE, M.D., and W. BURTON AYRE, M.D.

From the Department of Obstetrics and Gynecology, Royal Victoria Hospital, and the Cytological Laboratories, McGill University, Montreal, Canada

Behavior of a lesion constitutes the final criterion of diagnosis. For over 50 years pathologists have identified lesions as malignant on the basis of their behavior pattern as revealed by tissue biopsy. Hansemann,[6] however, as early as 1892 emphasized the morphologic characteristics of the cell as a sole and sufficient criterion of malignancy. The recent advent of the Papanicolaou technics[8–10] has focussed anew the attention of numerous investigators upon individual cell characteristics as evidence of malignant disease. By the application of these technics in vaginal cytology, serial studies in subjects having cells of abnormal morphology are readily possible. This report presents the cytologic and histologic features of one such case. Epithelial smears from the uterine cervix showed apparent progression from an abnormal or "precancer" cell type to cells representative of actual malignancy.

The method of scraping cells from the surface of the uterine cervix,[2] the so-called "surface-biopsy" of cells from the squamocolumnar region, is one modification of the Papanicolaou technic. Cervical cancer arises in a known accessible area, the squamous margin of the squamocolumnar junction.[7, 12, 13] Schiller,[11] with his vast experience in this field, is quite dogmatic in stating that 100 per cent of squamous cancers arise at this point. The surface-scraping technic consists in scraping the cells from the squamous marginal area as a method of detecting early evidence of malignant change. After studying many thousand samples of cells from this area using this technic, we have classified cells into the following four general types: (1) normal, (2) inflammatory, (3) "precancer"[5] and (4) cancer.

There has been much controversy concerning the designation as "precancer" cells of certain borderline or suspicious cells scraped from this area. This is to be

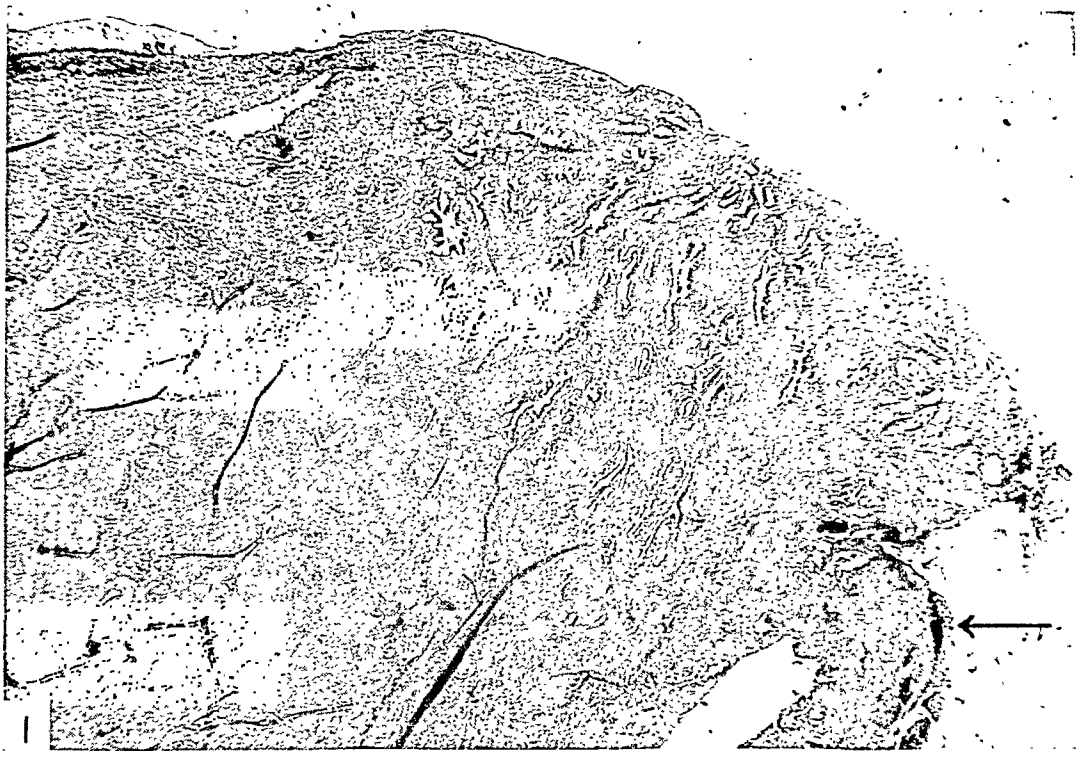
* This work was supported by funds from the Public Health Foundation for Cancer and Blood Pressure Research, Inc., Stamford, Connecticut.

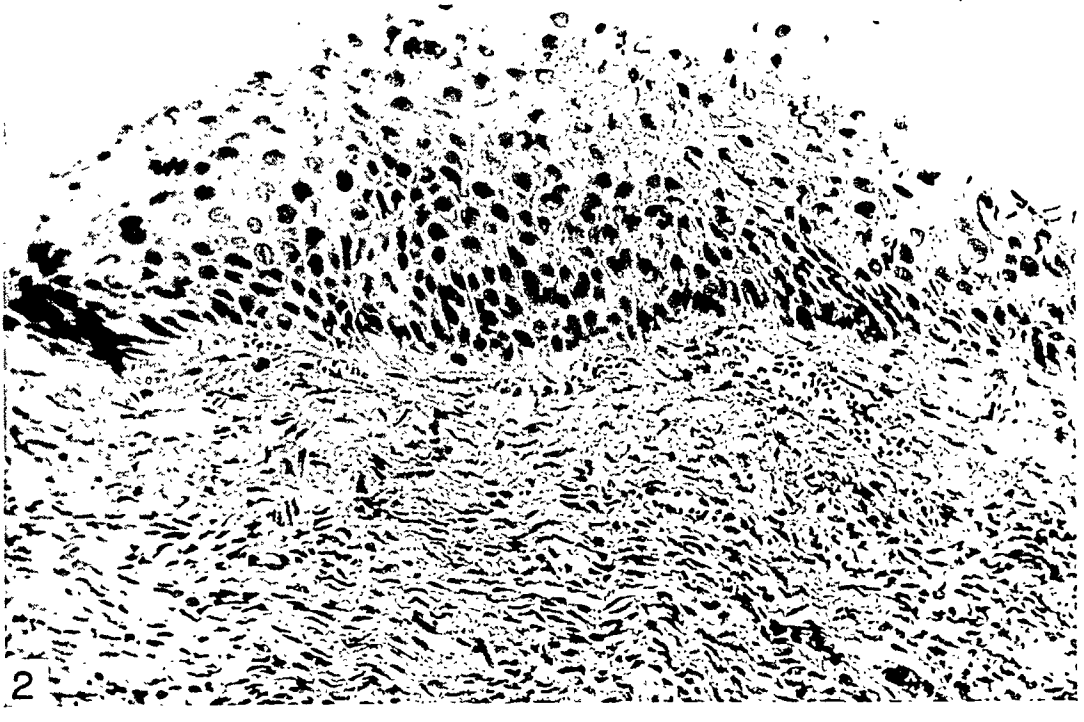
These studies were presented in a paper before the American Association for the Study of Neoplastic Diseases, Washington, D. C., April 8, 1949.

Received for publication, May 9, 1949.

Fig. 1. First biopsy of cervix was made in October 1948. The diagnosis was chronic cervicitis with erosion. A higher magnification of the area indicated by the arrow is shown in Figure 2. This is the only area in which any degree of abnormal cell change was demonstrable although multiple sections were cut. For five months preceding this cervical biopsy, repeated surface-cell scrapings consistently revealed innumerable cells and cell clusters diagnosed cytologically as "precancer" cells. × 15.

Fig. 2. Higher power of area indicated by arrow in Figure 1, showing some "cell atypism". × 100.





FIGS 1-2

expected in view of our scant knowledge of the factors that influence cells or that are associated with the metamorphosis of cells to a malignant state. The term "precancer" arouses antagonism in the minds of certain pathologists, and an alternative has been sought. The term "nearo-cancer" was first suggested to the author by Dr. Alexander Symeonides and Dr. Harold J. Stewart in a discussion at the National Cancer Institute. This term indicates the "earliest stage", and it is our belief that this stage, the "nearo-carcinoma" stage, of growth may be a precurser to the entity called "carcinoma *in situ*" or "intra-epithelial carcinoma".

Since behavior constitutes the ultimate criterion of diagnosis, we have been studying the behavior of cells from the same subjects during the past four years, and we are gradually gaining new knowledge concerning this controversial stage of cellular growth. Many years will probably elapse before we learn to understand the transitional stages in the development of a malignant process. In the present case an unusual opportunity was afforded to study, both by cytologic and by histologic technics, different stages of the growth process. The combined cytohistologic studies would appear to indicate relatively rapid progression in this case, from a cytologic stage diagnosed as "precancer" to actual carcinoma.

REPORT OF CASE

Mrs. S., age 32, para 3, was first seen by us in June, 1948, when she attended our free gynecologic clinic in the Royal Victoria Hospital. This patient had no specific gynecologic complaint, but stated that since the birth of a dead-born infant two years previously, she had not felt well and complained of irritability. The cervix appeared normal, but the routine surface-scraping from the squamocolumnar region of the cervix, which is taken from every new clinic patient, revealed cells of the "precancer" type (nearo-carcinoma cells). The surface-scraping cytologic test was again reported as "precancer" in July 1948 and also in September 1948. In October the patient was admitted to the hospital and tissue was excised from the cervix and curetted from the endometrium for biopsy. Multiple sections were cut from the cervical tissue (Fig. 1). An excerpt from the pathologic report reads: "Sections of cervix show with the exception of 2 or 3 areas, almost complete loss of all layers of the squamous epithelium. There is a marked infiltration of the subepithelial layer with red blood cells. There is a fibroblastic proliferation in the same area. There are 3 or 4 places where the squamous epithelium is forming. This seems somewhat hyperplastic. No evidence of malignancy. The glands are normal. Anatomic summary: Cervix, healing erosion, squamous hyperplasia."

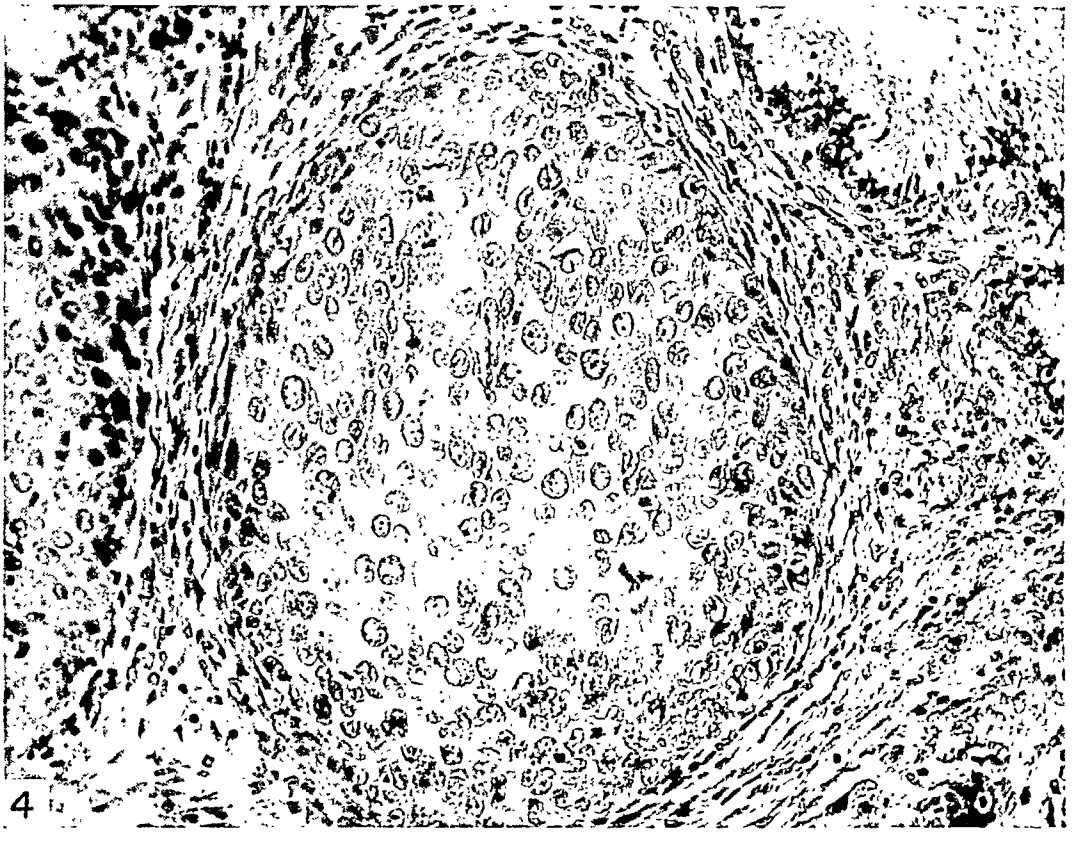
Careful study of the sections revealed one tiny area of "cell atypism" illustrated under high power magnification in Figure 2. The patient was discharged from the hospital without further treatment, but careful follow-up was continued. During the course of the next five months, the character of the abnormal cells in the cervical cell-scrapings was investigated from various aspects. Histochemical studies revealed that, unlike normal cells, they were persistently low in glycogen content.

In an attempt to promote reversion to normal, efforts were directed toward overcoming this cytoglycopenia. The action of estrogen and progesterone in promoting the deposition of

FIG. 3. Photomicrograph of tissue from surface removed for biopsy six months after the first biopsy and 10 months after first surface-cell scraping which was diagnosed as showing "precancer" cell changes. × 200.

FIG. 4. High power of one area of lesion seen in Figure 3, showing evidence of apparent infiltration. Observe well marked anaplasia and mitotic figures of squamous carcinoma. × 350.





FIGS. 3–4

glycogen in the normal squamous cells of the vaginal and cervical epithelium is well known. A therapeutic trial on these reagents, therefore, was given. From November 4, 1948 to December 6, 1948, a total of 480 mg. of progesterone was given intramuscularly in 20 mg. doses at fairly regular intervals. From December 10, 1948, to February 10, 1949, a total of 1500 mg. of stilbestrol was given intramuscularly beginning with 10 mg. doses daily and gradually increasing to a maximum of 50 mg. daily. In addition, throughout the period from November 4, 1948 to March 21, 1949, large doses of vitamin B complex were given orally and intramuscularly. During this period of five months, 60 cytologic cervical scrapings were taken at frequent intervals. The glycogen content of the cells was not influenced by therapy. Morphologic cell studies indicated progression apparently to an actual carcinoma. The cervix, meanwhile, still exhibited an unchanged normal gross appearance and there were no symptoms, such as bleeding or discharge, suggestive of carcinoma. Indeed, cervical scrapings with the wooden spatula (Fig. 6) failed to produce any subsequent traumatic oozing of blood which frequently occurs when clinically malignant tissue is scraped with the spatula. Cervical biopsies were again secured on March 20th in the Woman's Pavilion, Royal Victoria Hospital, and in view of the absence of any focal lesion of suspicious character, the entire ring of tissue comprising the squamocolumnar junction (Figs. 7 and 8) was excised.[4] Following the excision of this cone of cervical tissue, electroconization was employed to remove an additional cone of endocervical tissue. This procedure performs the function of sealing off both lymphatics and blood vessels against possible spread of cancer cells, while the excision of the extra cone of endocervical tissue is believed to have further therapeutic value.

A "ring-section" presents the pathologist with adequate tissue cleanly excised (and not cooked and destroyed by the high frequency current), permitting multiple blocks to be made from the four quadrants of the squamocolumnar circumference. Indeed, the tissue is available for serial sections if this is found necessary or desirable. Figures 3 and 4 show an island of tissue which appears to indicate definite infiltration.

An excerpt from the pathologic report reads: "This squamous epithelium is hyperplastic and there is in several areas evidence of basal cell hyperplasia. In addition, there is loss of normal polarity with anaplasia of the squamous epithelium in many places. There are a few mitoses in the superficial third of the epithelium. There is marked tonguing of the basal layers and invasion of the glands with this same tissue. Anatomic summary: Endometrium, hyperplasia; Cervix, carcinoma *in situ*, 'early invasion'."

Following careful study of the tissues and discussion with a number of gynecologists of the Royal Victoria Hospital staff, it was decided to treat this patient by surgical extirpation. Accordingly, in April 1949 a panhysterectomy was performed and serial sections were then made of the removed cervix. No further malignant tissue was identified, indicating that the "ring-excision" of the cone

FIG. 5. Clinical appearance of the cervix preceding second removal of tissue for biopsy. Observe absence of any clinical lesion to arouse suspicion of carcinoma.

FIG. 6. Diagrammatic representation of surface-scraping of cervix. (1) Arrow points to squamocolumnar junction of the cervix. (2) Arrow points to endocervical epithelium. Technic for cervical scraping. The cervical scraper collects cells from the spot where tissue is surgically removed for biopsy. It scrapes the squamous margin, in which area cancer is prone to develop. Two scrapings are taken from each patient. The cervical mucus adheres better to the wood than to glass. The first scraping removes the mucus-plug and the cells which have been exfoliated into it. The second scraping scrapes cells and cell clusters from the tissue below the level of exfoliation. The cells are fresh and their origin is known. The cell clusters from the surface scraping furnish material for a most sensitive test to permit evaluation of abnormal growth in the cells.

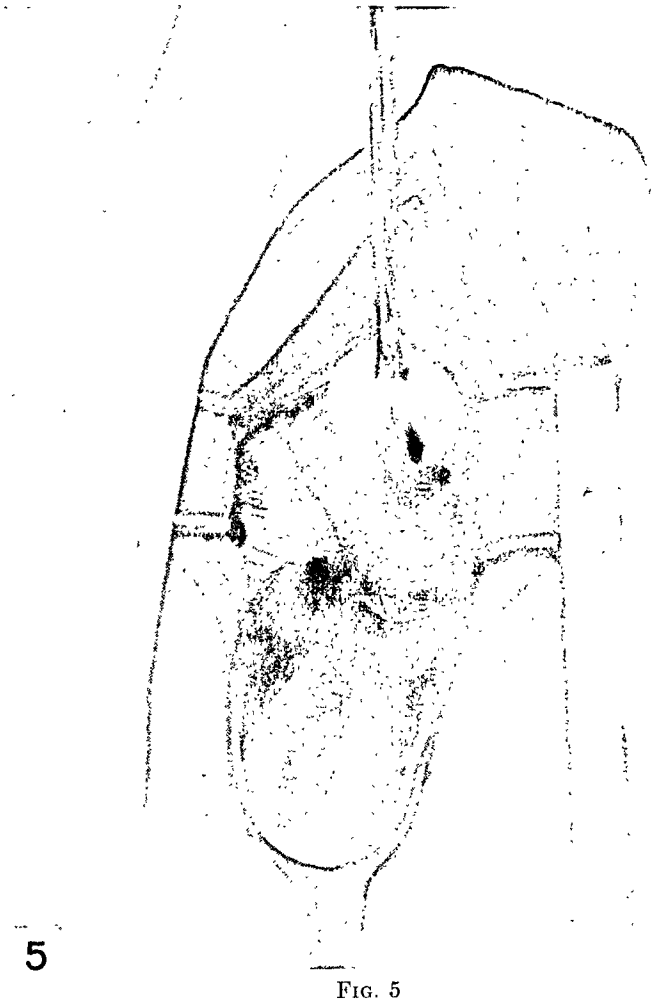


FIG. 5

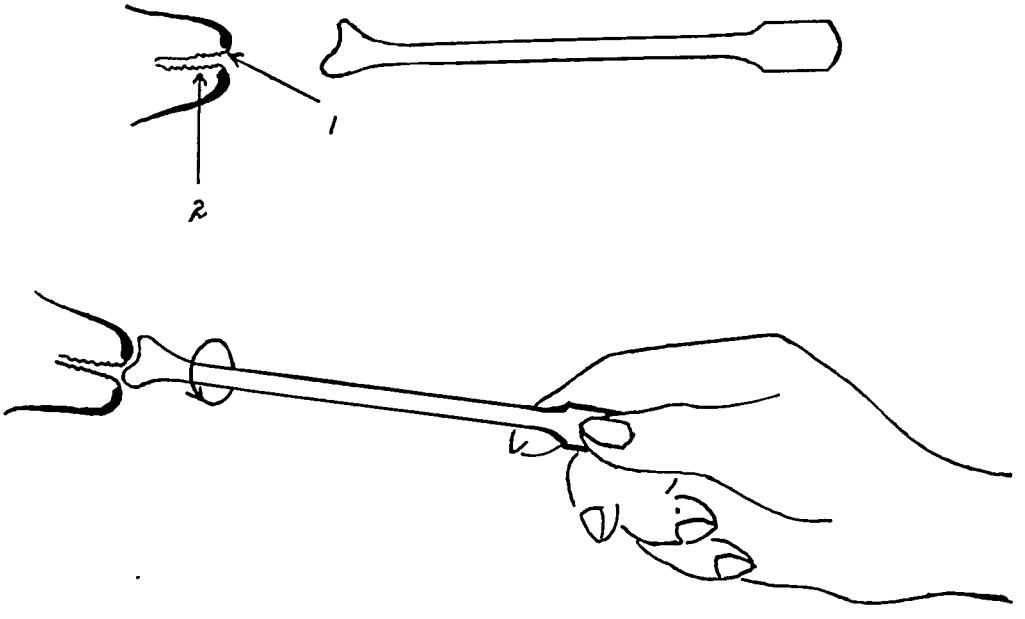


FIG. 6

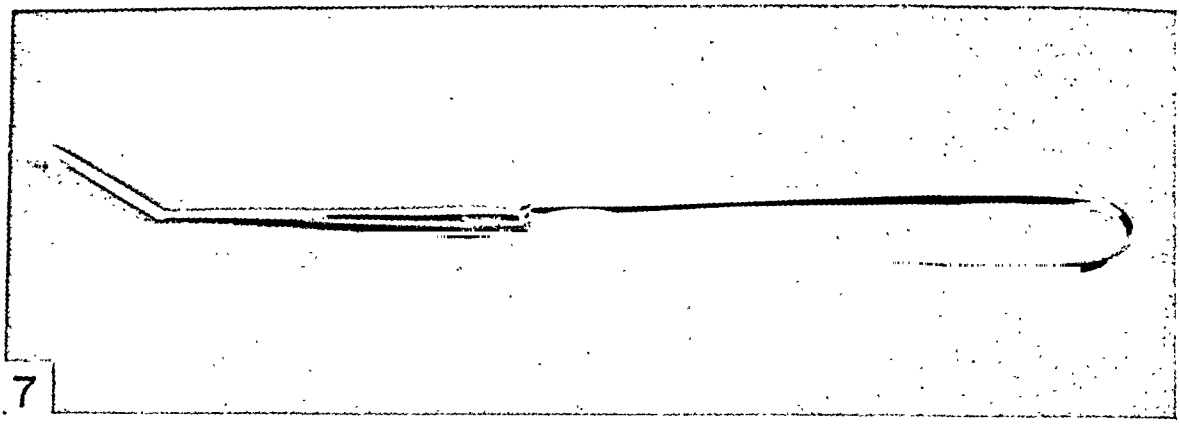


FIG. 7. Cervical cone-knife (senior author's design) for securing ring-section from squamocolumnar junction of the cervix.

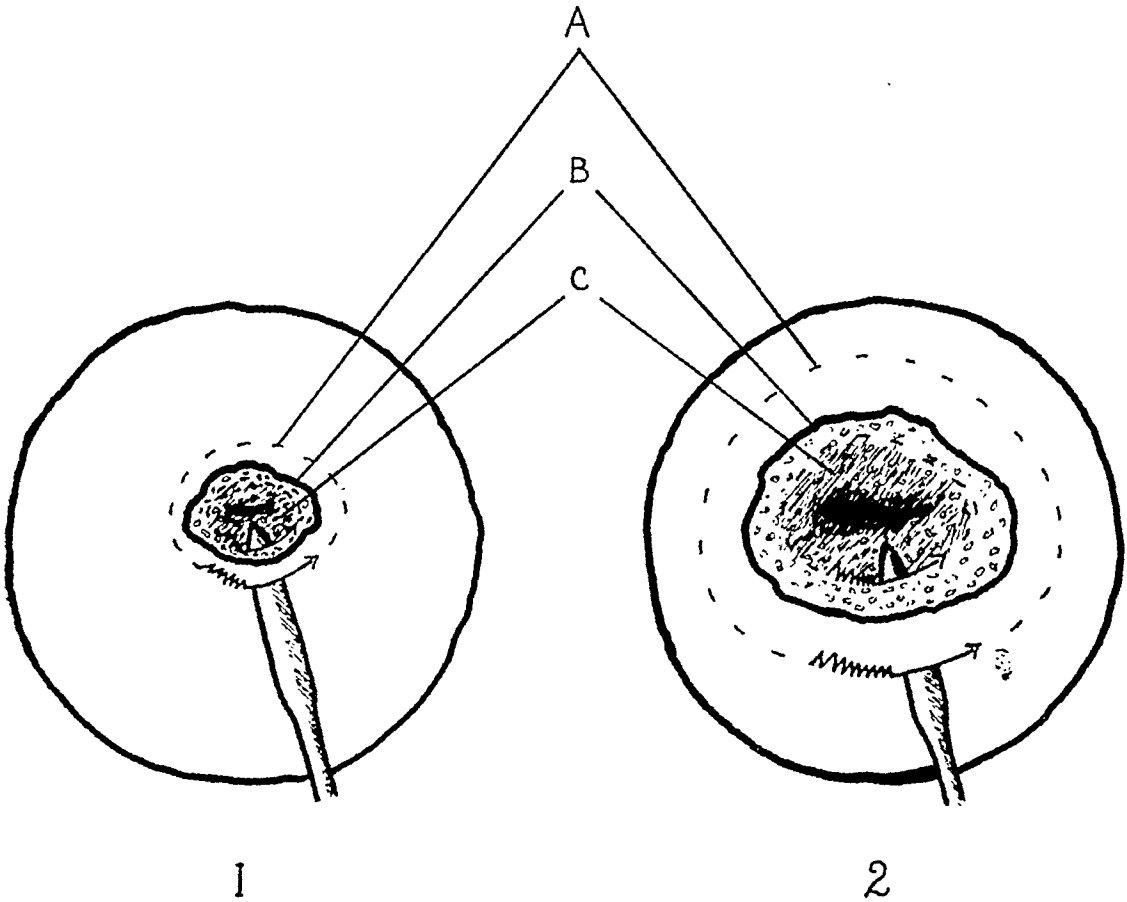


FIG. 8. Diagrammatic representation of uterine cervix with cone knife in operation. (1) Normal-appearing type, showing small squamocolumnar circumference near cervical os. (2) Eroded type, showing large squamocolumnar circumference. A, endocervical glandular epithelium, B, margin of squamous epithelium (squamocolumnar junction) and C, squamous epithelium of cervix. The pointed blade with the dual razorsharp edges is inserted into the cervix 0.5 cm. distal to the margin of the squamous epithelium, being directed toward the center of the cervical circumference. A circular "ring" of tissue is excised, removing the entire squamocolumnar junctional zone for biopsy. Preclinical squamous cervical cancer will be found, if present, in the clear nonstippled portion of the excised collar of tissue.

of tissue embracing the squamocolumnar region had apparently removed the entire neoplasm.

It is not proposed in this preliminary paper to present in detail the morphologic features and characteristics of cells designated as "precancer" or "nearo-cancer" cells. To demonstrate these cells adequately, their true microscopic appearance is better presented by color photomicrographs. These will be published at a later date along with a follow-up of a larger group of cases in which periodic studies were made over a three-year period. It perhaps is not remarkable that progression to early carcinoma was demonstrable in this case though the rate of progression is doubtless extremely variable in different cases of cervical cancer. It is of greater interest, however, to emphasize that a sensitive method of identifying the early formative stage of cervical cancer is now within our reach.

SUMMARY

A case is presented in which abnormal, so-called "precancer" cell types in epithelial smears from the uterine cervix were studied at frequent intervals within one year. Progesterone and stilbestrol were administered for a three-month period during this time in an effort to promote normal cell development, without success. The abnormal cells exhibited morphologic progressive change to a malignant type, following which biopsies of cervical tissue confirmed the presence of carcinoma in an early infiltrative stage. Clinically, the cervix remained grossly normal in appearance throughout the period of observation.

It is the opinion of the writers that the cervical cell-scraping modification of the Papanicolaou technic constitutes a useful and highly sensitive method of studying the morphology of cervical epithelial cells. These studies in cervical cytology are bringing to light more and more evidence in support of the statement made by the illustrious James Ewing: "It is not true that a pathological condition must be either cancer or not cancer. It may be neither one nor the other. It may be in the process of becoming cancer."

Acknowledgments. Grateful appreciation is expressed for hormonal and pharmaceutical preparations used in this study. Progesterone was prepared especially for experimental use by Ayerst, McKenna & Harrison Limited, Montreal; Stilbestrol dipropionate was especially prepared by Charles E. Frosst & Company, Montreal; and supplies of vitamin B complex were generously provided by Lederle Laboratories, Division of American Cyanamid Company, New York. The photomicrographs were made by Harold Coletta, McGill University, Montreal.

REFERENCES

1. AYRE, J. E.: A simple office test for uterine cancer diagnosis. Canad. M. A. J., **51**: 17–22, 1944.
2. AYRE, J. E.: Vaginal and cervical cytology in uterine cancer diagnosis. Am. J. Obst. and Gynec., **51**: 743–749, 1946.
3. AYRE, J. E.: Selective cytology smear for diagnosis of cancer. Am. J. Obst. and Gynec., **53**: 609–617, 1947.
4. AYRE, J. E.: Diagnosis of preclinical cancer of the cervix. J. A. M. A., **138**: 11–13, 1948.
5. AYRE, J. E.: The vaginal smear: "precancer" cell studies using a modified technique. Am. J. Obst. and Gynec., in press.
6. HANSEMANN, D.: Studien über die Spezefizität den Altruismus und die Anaplasia der Zellen mit besonderer Berücksichtung der Geschwülste. Berlin: A. Hirschwald, 96 pp., 1893.

7. Novak, E.: Pseudomalignant and precancerous lesions of the cervix. J. A. M. A., **108**: 1145–1151, 1937.
8. Papanicolaou, G. N.: New cancer diagnosis. Proc. Third Race Betterment Conf., p. 538, 1928.
9. Papanicolaou, G. N., and Traut, H. F.: The diagnostic value of vaginal smears in carcinoma of the uterus. Am. J. Obst. and Gynec., **42**: 193–206, 1941.
10. Papanicolaou, G. N., and Traut, H. F.: Diagnosis of Cancer by Vaginal Smears. New York: The Commonwealth Fund, 1943.
11. Schiller, W.: Personal communication to the senior author.
12. Taylor, H. C., Jr., and Guyer, H. B.: A seven-year history in early cervical cancer. Am. J. Obst., and Gynec., **52**: 451–455, 1946.
13. TeLinde, R. W., and Galvin, G.: The minimal histological changes in biopsi s to justify a diagnosis of cervical cancer. Am. J. Obst. and Gynec., **48**: 774–794, 1944.

FURTHER STUDIES ON THE rh^w FACTOR*

A. S. WIENER, M.D., L. J. UNGER, M.D., AND C. A. MAZZARINO, B.A.

From the Serological Laboratory, Office of the Chief Medical Examiner, New York, and the Blood and Plasma Bank of the New York University-Bellevue Medical Center, New York, New York

In a previous article[10] the results of investigations of a new variant of the Rh factor, rh^w, were described. This factor had originally been discovered by Callender, Race and Paykoc.[1] The purpose of the present report is to describe the results of further studies on the rh^w factor.

In our previous study, confirming the observations of Callender *et al.*, we found that anti-rh^w serum clumped only bloods of types Rh_1 and Rh_2.† This indicated that rh^w is a partial antigen within the structure of the Rh_1 agglutinogen. The question then arose whether similar variants exist among bloods of type rh′ and type rh_y.‡ In order to settle this point, a series of bloods of type rh′ have been tested with anti-rh^w serum and in the present paper the results of this study will be reported.

MATERIALS AND METHODS

The blood specimens examined were obtained from donors who presented themselves at the Blood and Plasma Bank of the New York University–Bellevue Medical Center. The bloods of these donors were first screened with Rh_0 slide-test conglutinating serum. All bloods reacting negatively with this serum were further tested by the tube-agglutination method with anti-Rh_0, anti-rh′, and anti-rh″ serums. All bloods which proved to belong to type rh′ or type rh_y were then subjected to a complete retesting with serums of specificities anti-Rh_0, anti-rh′, and anti-rh″ by the tube-agglutination method, and in addition were tested with serum of specificities anti-hr′ and anti-rh^w, and some of the specimens were also tested with anti-hr″ serum. All bloods were also tested for their A-B-O groups and subgroups and for the M-N types. A total of 145 specimens were examined for the rh^w factor, of which 120 were derived from members of the Caucasoid race and 25 from the Negroid race.

RESULTS

Not one of the 145 bloods tested was clumped by the anti-rh^w serum. A list of the blood types of the specimens examined and their racial derivation is given in Table 1. Based on the usual incidence of 1 per cent for type rh′ among Caucasoids and the frequency of approximately 3 per cent among Negroids, it will be seen that the series examined represents a study of a random group of approximately 12,000 Caucasoid and 750 Negroid persons.

* Received for publication, May 2, 1949.

† Blood of phenotype Rh_2 was formerly designed as Rh_1Rh_2 (cf. Wiener[9]).

‡ Blood of phenotype rh_y was formerly designated as rh′rh″.

TABLE I

LIST OF BLOODS TESTED FOR rh^w FACTOR

CAUCASOIDS				NEGROIDS
1. ONrh'rh	31. OMrh'rh	61. OMrh'rh	91. OMNrh'rh	1.*A_1Mrh'rh
2. BMNrh'rh	32. OMNrh'rh	62. A_1MNrh'rh	92. BMrh'rh	2. A_1Nrh'rh
3. A_1MNrh'rh	33. OMrh'rh	63. A_1Nrh'rh	93. OMNrh'rh	3. ONrh'rh
4. A_1MNrh'rh	34. A_1MNrh'rh	64. A_1MNrh'rh	94. BMrh'rh	4. OMNrh'rh
5. ONrh'rh	35. A_2Mrh'rh	65. OMNrh'rh	95. A_1Nrh'rh	5. ONrh'rh
6. OMNrh'rh	36. A_1MNrh'rh	66. A_1MNrh'rh	96. OMNrh'rh	6. BMNrh'rh
7.*OMrh'rh	37. OMrh'rh'	67. OMNrh'rh	97. A_1Nrh'rh	7. A_1MNrh'rh
8. A_1MNrh'rh	38. BNrh'rh	68. ONrh'rh	98. A_1MNrh'rh	8. A_1Mrh'rh
9. OMrh'rh	39. OMNrh'rh	69. A_1MNrh'rh	99. ONrh'rh	9. OMrh'rh
10. BMrh'rh	40. A_1MNrh'rh	70. OMrh'rh	100. A_1Mrh'rh	10. A_2BMNrh' rh
11. A_1MNrh'rh	41. BMNrh'rh	71. ONrh'rh	101. OMNrh'rh	
12. OMrh'rh	42. OMNrh'rh	72. OMNrh'rh	102. A_1MNrh'rh	11. BMrh'rh
13. A_2MNrh'rh	43. A_1MNrh'rh	73. OMNrh'rh	103. A_1MNrh'rh	12. BMNrh'rh
14. A_1Mrh'rh	44. BMNrh'rh	74. A_1Mrh'rh	104. ONrh'rh	13.*A_1Nrh'rh
15. A_1MNrh'rh	45. ONrh'rh	75. A_1Mrh'rh	105. OMrh'rh	14. A_1BNrh'rh
16. A_1MNrh'rh	46. BMrh'rh	76. OMrh'rh	106. BMNrh'rh	15. A_1Mrh'rh
17. A_1MNrh'rh	47. BMNrh'rh	77. BMNrh'rh	107. ANrh'rh	16. OMNrh'rh
18. OMNrh'rh	48. A_1MNrh'rh	78. OMrh'rh	108. OMNrh'rh	17. A_1MNrh'rh
19. BMrh'rh'	49. A_1MNrh'rh	79. BMrh'rh	109. OMrh'rh	18. BMNrh'rh
20. OMrh'rh	50. OMNrh'rh	80. BMrh'rh	110.†BMrh$_y$rh	19. ONrh'rh
21. A_1Mrh'rh	51. OMNrh'rh	81. A_1BMrh'rh	111. A_1Mrh'rh	20. A_1Mrh'rh
22. OMrh'rh	52. OMNrh'rh	82. BMrh'rh	112. A_1Mrh'rh	21. BNrh'rh
23. OMNrh'rh	53. BMNrh'rh	83. BMNrh'rh	113. OMNrh'rh	22. BMrh'rh
24. OMrh'rh	54. A_2BMNrh'rh	84. ONrh'rh	114. OMrh'rh	23. A_1MNrh'rh
25. OMNrh'rh	55. A_1Mrh'rh	85. A_1MNrh'rh	115. A_1BMrh'rh	24. OMrh'rh
26. $A_{1,2}$MNrh'rh	56. OMrh'rh	86. OMNrh'rh	116. A_1MNrh'rh	25. A_1MNrh'rh
27. BMNrh'rh	57. OMrh'rh	87. A_1MNrh'rh	117. BMrh'rh	
28. OMNrh'rh	58. OMrh'rh'	88. OMNrh'rh	118. AMNrh'rh	
29. A_1MNrh'rh	59. OMrh'rh	89. A_2MNrh'rh	119. OMNrh'rh	
30. A_1Mrh'rh	60. A_2BMrh'rh	90. A_1MNrh'rh	120. A_1MNrh'rh	

* These bloods gave weak or "intermediate" reactions with anti-rh' serum.

† Type rh$_y$rh indicates blood which belongs to type rh'rh" and also contains factors hr' and hr".

DISCUSSION

When the existence of more than one Rh factor was originally demonstrated by Wiener,[3] all the theoretical possibilities concerning their genetic transmission were considered, namely, heredity by independent pairs of genes, inheritance by linked genes, and inheritance by a series of allelic genes. It was readily demonstrated that the observations precluded the existence of separate pairs of allelic genes for each of the pairs of Rh-Hr factors and, therefore, the theory of multiple allelic genes was adopted by Wiener. At the present stage of the work, the existence of a series of at least nine sharply defined genes has been clearly established, namely, r, r', r'', r^y, R^0, R^1, R^2, R^z, and R^{1w}. The results of the study described here indicate that gene r'^w either does not exist at all or is extremely rare. Other theoretically possible genes which might be searched for in connection with the rh^w factor are genes R^{zw} and r^{yw}.

Unfortunately, British investigators[2] have adopted the concept of linked genes which had formerly been disproved and discarded by Wiener, and have made this the basis for an unnecessary duplicate system of notations for the Rh-Hr factors. The factors Rh_o, rh′ and rh″ are designated as D, C, and E, respectively, by the British workers, while the factors Hr_o, hr′ and hr″ are designated as d, c, and e, respectively. Genotype R^1r would be written as genotype CDe/cde by the British workers. The British notations make no provision for phenotype designations. According to Fisher, factor rh′ arose from type Rh_1 blood by crossing over. Thus, while genotype CDe/cde is considered to yield ordinarily gametes, half of which bear CDe and half of which bear cde, by crossing over, gametes Cde and cDe could result. However, if this concept were correct, then blood of rh′^{w} should have arisen in a similar way by crossing over from blood of type Rh_1^w (genotype C^wDe/cde), and the frequency of positive reactions with anti-rh^w serum among individuals of type rh′ should be about the same as among individuals of type Rh_1. But this is not the case, as shown by the present study. The present study thus provides additional evidence refuting the concept of triple linked genes to account for the hereditary transmission of the Rh-Hr blood factors.

SUMMARY

A series of 120 Caucasoid and 25 Negroid persons of type rh′ and one additional Caucasoid individual of type rh′rh″ were tested with anti-rh^w serum. Not one of the blood specimens was clumped by this serum. This finding provides further evidence refuting the concept of triple linked genes and supporting the theory of multiple allelic genes to account for the hereditary transmission of the Rh-Hr blood factors. In the present state of knowledge, it is surprising to us to find that there still are some competent workers who use the unnecessary and confusing CDE notations on the ground that they are "simpler". Certainly, the incorrect name "small c, small d, small e over small c, small d, small e" is not simpler than the precise designation "type rh". In addition, the evidence presented here and in previous articles[4–8,10] proves that the CDE notations are based on incorrect serologic and genetic concepts, and in scientific work accuracy should take priority over simplicity.

Acknowledgment. The authors are indebted to Dr. Birger Broman who provided the potent anti-rh^w serum which made this study possible.

REFERENCES

1. CALLENDER, S. T., RACE, R. R., AND PAYKOC, Z. V.: Hypersensitivity to transfused blood. Brit. M. J., **2**: 83–84, 1945.
2. FISHER, R. A.: Cited by Race, R. R.: An "incomplete" antibody in human serum. Nature, London, **153**: 771–772, 1944.
3. WIENER, A. S.: Blood Groups and Transfusions. Ed. 3. Springfield, Ill.: Charles C Thomas, p. 254, 1943.
4. WIENER, A. S.: Theory and nomenclature of the Rh types, subtypes and genotypes Brit. M. J., **1**: 982–984, 1946.
5. WIENER, A. S.: The Rh system in the chimpanzee. Science, **104**: 578–579, 1946.
6. WIENER, A. S.: Nomenclature of the Rh factors. Lancet, **1**: 343, 1948.
7. WIENER, A. S.: Anti-Rh serum nomenclature. Brit. M. J., **1**: 805–806, 1948.
8. WIENER, A. S.: The Rh factor. Brit. M. J., **1**: 404–405, 1949.
9. WIENER, A. S.: An Rh glossary. Dade County Med. Assoc. Bull., **20**: 20–32, 1949.
10. WIENER, A. S., AND GORDON, E. B.: Studies on the blood factor rh^w. Am. J. Clin. Path., **19**: 621–629, 1949.

ABNORMAL AGGLUTINABILITY OF RED CELLS IN PYOGENIC INFECTIONS

Report of Two Cases*

G. ENGLESON, M.D., AND R. GRUBB, M.D.

From The Pediatric Clinic and The Bacteriologic Institute, University of Lund, Sweden

We have observed the rare phenomenon of abnormal agglutinability of the red cells in 2 patients infected with pyogenic microorganisms. In Case 1 the patient's blood group seemed to alter. In Case 2 auto-agglutination was manifest at body temperature and the clinical picture and the autopsy findings throw some light upon the physiopathologic significance of the phenomenon.

REPORT OF CASES

Case 1

A previously healthy 28 year old woman became febrile (39–40 C.; 102.2–104.0 F.) in the puerperium of her fourth normal delivery. Sulfathiazole was given, the temperature dropped to 38 C. (100.4 F.) and remained at this level for two months. Large tender swellings developed in the parametria. After the patient had been ill for two months she suddenly presented the picture of ileus. At operation the distal portion of ileum was found adherent in the pelvis and the adhesions were severed. For 10 days following the operation the patient was seriously ill with high fever (39–40 C.) and general malaise. Thereafter the temperature gradually returned to normal, and she was finally considered to be well and was released.

In the two-month period following the delivery the patient was given three blood transfusions without untoward reactions. She belonged to blood group B Rh+. At all three transfusions the patient's serum and red cells were cross-matched with the cells and serum of group B donors and no agglutination was observed. Ten days after the operation for ileus, when another transfusion was planned, it was reported from the ward that the patient now apparently belonged to blood group AB, as her red cells were agglutinated by anti-A and anti-B serums. Closer examination of the patient's blood on this day gave the following results: At room temperature the red cells (washed twice in saline) were agglutinated by three anti-A and three anti-B serums, by the serums from two persons of group A_1B and one of group A_2B, and by the patient's own serum. At 37 C. the agglutination was weaker and the patient's serum did not agglutinate her own cells. The patient's serum did not agglutinate normal O or B cells at room temperature. The titer of the alpha agglutinins in her serum was 1:128 for A_1 cells and 1:32 for A_2 cells. The titer of cold agglutinins (tested against O cells overnight at +4 C.) was 1:8. One of the group AB serums which had an original agglutination titer of 1:16 against the patient's cells, was absorbed by the latter and the agglutinins were wholly removed. On the next day these tests were repeated on a fresh sample drawn under sterile conditions with the same results. The results were similar on several occasions during the following month. Six weeks

* Received for publication, April 18, 1949.

after the abnormal agglutinability had first been observed the patient's red cells exhibited the same properties as do normal cells of group B.

Case 2

A 9 year old boy, previously healthy, fell ill on December 24, 1947 with bullous eruptions on the fingers and toes. Two days later the vesicles became purulent and some broke. On December 29 there were generalized hemorrhages into the skin. His general condition deteriorated considerably and on December 30 he was admitted to the Pediatric Clinic. At this time he was distressed, somnolent and his skin was cold and clammy. His temperature was 38.7 C. (101.7 F.), pulse rate 110, and blood pressure 115/75 mm. Hg. There were generalized petechial hemorrhages into the skin, especially on the forearms and face. The fingers and toes exhibited extensive purulent, edematous and bullous changes. The spleen was palpated immediately below the costal arch and was somewhat tender. The liver was not enlarged. The heart and lungs were normal. Neurologically, there were no unusual findings. Ophthalmoscopic examination revealed hemorrhages and dilated blood vessels.

Course. The pyodermic processes on the fingers and toes responded readily to local treatment and healed, but the hemorrhages into the skin persisted unchanged. The patient was treated immediately with penicillin and sulfathiazole but without substantial response and

TABLE 1

TITERS OF IRREGULAR AGGLUTININS IN CASE 2

CELLS TESTED	TITER AT DESIGNATED TEMPERATURES		
	37 C.	18 C.	4 C.
Patient's own cells	1:128	1:256	1:400
A_1 cells	1:2	1:32	1:400
A_2 cells	1:2	1:32	1:400
O cells	1:2	1:32	1:400

streptomycin was, therefore, also administered. A few days after the commencement of streptomycin therapy, the patient's temperature returned to normal but his shock-like condition persisted. On January 6, 1948 the patient suffered a brief convulsive seizure. On January 11 he had another attack of convulsions with severe jerkings of the right arm and leg. He was then intermittently unconscious until his exitus on this day.

Serologic findings. December 30, 1947: Auto-agglutination occurred in the blood specimen even after five hours at 37 C.; a homogeneous suspension of red blood cells was unobtainable; agglutination titer in the cold, 1:500 (group O cells, 4 C.). January 2, 1948: Agglutination still strong at 37 C.; cells were washed 12 times with a warm (50 C.) solution of normal saline, after which a homogeneous suspension was obtained. In grouping tests performed at 37 C., the patient's cells rapidly formed one large clump with two different anti-A serums, were faintly but clearly flocculated by three anti-B serums, by A_1B serum, and by the patient's own serum; group B cells agglutinated readily and completely in the patient's serum; A_1, A_2, and O cells, as well as patient's own cells, flocculated slightly and more slowly. M, N, A_2, and P determinations were performed without difficulty. A reliable Rh determination could not be made because the only serums obtainable were those of human beings, which in this case would agglutinate the patient's cells. Interpretation: The patient was assignable to group A_1NP.

At 18 C. the patient's serum on glass slides agglutinated blood cells from 10 persons belonging to group A, and cells from 6 persons belonging to group O. The titer of the irregular agglutinins in the patient's serum was determined, the incubation period being two hours, and normal saline being used as diluting fluid. The results of this examination will be apparent from Table 1. The patient's red cells were tested at 18 C. with three different serums from donors belonging to group AB, and clumping was observable with dilutions of 1:64, 1:8 and 1:16, respectively.

On January 8, strong auto-agglutination (titer 1:128) was still present at 37 C. The irregular agglutinins, that were present in the patient's serum and reactive against foreign cells, were absorbable by group O cells. After absorption the beta agglutinins remained in the patients's serum. Owing to technical difficulties, it was not possible to draw off a sufficient quantity of blood to perform absorption experiments with the patient's own cells.

TABLE 2

LABORATORY FINDINGS IN CASE 2

DATE	HEMOGLOBIN (SAHLI)	ERYTHROCYTE COUNT*	LEUKOCYTE COUNT	PLATELET COUNT	RETICULOCYTE COUNT	NON-PROTEIN NITROGEN	SEDIMENTATION RATE* (LANDAU)	BILIRUBIN
	per cent				*per 1000 RBC*	*mg./100 ml.*		
Dec. 30	43	1,820,000?	13,800	254,000	39	—	—	—
Jan. 2	34	—	15,300	185,000	42	40		
Jan. 8	25		13,800			40		0.8/ 200,000
Jan. 10	23	1,280,000?					44/45	

* On account of agglutination, the erythrocyte count and the erythrocytic sedimentation rate (which corresponds to 145 by Westergren's method) cannot be considered reliable.

December 30: Differential count, segmented neutrophils 41.5 per cent, stab cells 15.5 per cent, eosinophils 0.5 per cent, basophils 0, lymphocytes 18.5 per cent, monocytes 1 per cent, immature white cells 22 per cent, megakaryocytes 1 per cent; nucleated red cells 3.5 per cent. Bleeding time, 2 min. Coagulation time, 5½ min. The size of the erythrocytes could not be determined on account of agglutination. Urinalysis, no albumen. From December 30 to January 5, the urinary sediment was normal; from January 5 to 10, the urine contained many red cells and granular casts. Benzidine tests and examinations for hemoglobinuria were negative until January 6, after which date hemoglobinuria was demonstrable. Tests for urobilin and urobilinogen were negative and no porphyrins were demonstrable. Electrocardiograms: December 31, normal findings; January 7, split QRS complex, elevation of S-T segment in Lead I and inversion of T III. Bacteriologic cultures of blood on January 2 and 6, and of cerebrospinal fluid on January 7, revealed no growth. The Donath-Landsteiner test yielded no hemolysis. The Pal-Bunnell reaction was negative. The Wassermann and flocculation tests of cerebrospinal fluid were negative.

Autopsy findings. Septicemia. Acute splenic tumor. Pronounced hemosiderosis of kidneys, spleen and liver. Numerous agglutinates in pial vessels. Thrombosis of left internal carotid artery and fresh infarction in left cerebral hemisphere. Microscopic examination revealed blood pigment in liver and spleen and especially in tubular epithelium of kidneys. Many tubules contained pigmented thrombi in their lumens. The follicles of the spleen were

swollen and revealed mobilization of large histocytic and reticulo-endothelial cells. The thrombus in the internal carotid artery was nonseptic and exhibited an ordinary structure.

Recapitulation. A 9 year old boy fell ill with pyoderma and acute sepsis. Auto-agglutination was observed at 37 C. Despite intensive therapy with sulfathiazole and antibiotics, he expired. Autopsy revealed signs of intravital hemolysis and a thrombus in the left internal carotid artery.

DISCUSSION

The cases described are remarkable inasmuch as their red cells were agglutinated by normal human serums independent of alpha and beta agglutinins. This phenomenon is known to occur under the following conditions: (a) When the blood sample *in vitro* has been contaminated with certain bacteria (Hübener-Thomsen phenomenon). This occurrence is considered to be due to bacterial enzymes releasing a receptor in the blood corpuscles.[8] Normal serum contains absorbable agglutinins against this receptor. The production of hemagglutinating properties of serum by bacterial action has also been reported.[6] (b) When incomplete agglutinins ("blocking antibodies") are bound to the blood corpuscles.[20] In normal serum there is an unabsorbable fraction making a manifest agglutination possible in this case. (c) When red cells *in vitro* have been subjected to the action of certain viruses.[4]

The literature contains but few reports of cases in which the fresh blood cells exhibited abnormal agglutinability. A list of these together with our own 2 cases is given in Table 3.

It will be apparent from the table that abnormal agglutinability may be demonstrable under different conditions. Most of the cases listed were infected with pyogenic microorganisms. Furthermore, it is obvious that the degree of such agglutinability may vary from one case to another. The agglutinability in question is not bound exclusively to the blood group O, which seemed to be the case until as recently as 1946 (Boormann, Loutit and Steabben[3]). Judging by the table, it would seem as if abnormal agglutinability may be ascribed to different causal factors.

One is tempted to believe the abnormal agglutinability in the septicemic cases to be an intravital Hübener-Thomsen phenomenon. Those agglutinins appearing in normal serums, responsible for this phenomenon, are absorbable. Their titers vary in different serums and in inverse proportion to the temperature. Such phenomena were also observable in relatively many of the "infected" cases listed in Table 3.

The classical Hübener-Thomsen phenomenon is transmissible, it being possible to transfer bacteria from one tube to another. In the cases published by Boormann, Loutit and Steabben, transmissibility could not be demonstrated. This does not, however, argue against the abnormal agglutinability being an intravital Hübener-Thomsen phenomenon, because Friedenreich[8] showed that even sterile filtrates of bacterial cultures can produce such abnormal though nontransmissible agglutinability. Bacterial enzymes *in vivo* can, of course, affect the blood corpuscles despite a possible absence of bacteria from the blood stream.

It has been suggested[12] that the therapeutic agents used (serum, sulfa prepara-

tions) might have been responsible for the abnormal agglutinability but this can hardly apply to the 2 cases described above. In Case 1 the change occurred about one month after the termination of sulfa treatment, and in Case 2, the change was noticed before the commencement of any therapy whatsoever.

The 2 cases described showed auto-agglutination, as the patients' serums agglutinated their own blood corpuscles at room temperature.

In the vast majority of cases, auto-agglutination is caused by the appearance of auto-agglutinins in the serum. Such agglutinins have been demonstrated in primary atypical pneumonia, in acquired hemolytic anemia, and sporadically in many other conditions.[15, 16, 18] In our Case 1, auto-agglutination was due to a change in the blood cells, and in our Case 2, to a combination of changes in the blood corpuscles and in the serum. As a rule auto-agglutination is not manifest

TABLE 3

CASES REPORTED WITH ABNORMAL AGGLUTINABILITY OF RED BLOOD CELLS

INVESTIGATORS	DIAGNOSIS	BLOOD GROUP	AGGLUTINATING SERUM	AUTO-AGGLUTINATION AT 37 C.
Benhamou, Nouchy[2]*	Malaria, parotitic abscess	B	—	+
Levine, Katzin[12]	Sepsis. Pneumococcus type I	O	15%	O
Gaffney, Sachs[9]	Syphilis	O	89%	O
Gaffney, Sachs[9]	No disease	O	73%	O
Currie[5]*	Acute hemolytic anemia	O	—	O
Basil-Jones, Sanger, Walsh[1]	Puerperal sepsis	O	45/45	O
Boorman, Loutit, Steabben[3]	Incomplete abortion	O	38/39	O
Boorman, Loutit, Steabben[3]	Parametritis	O	142/143	O
Wasastjerna[19]	Subacute hemolytic anemia	A	—	+ at 33 C.
Engleson, Grubb	Parametritis	B	7/7	O
Engleson, Grubb	Septicemia	A	7/7	+

* Data insufficient

at body temperature and is generally to be considered as an *in vitro* phenomenon without physiopathologic significance. Some cases [13] of this type, however, show cold hemoglobinuria, a Raynaud syndrome[7, 13, 17] or paroxysmal hemoglobinuria with positive Donath-Landsteiner reaction.

Those cases in which auto-agglutination was manifest at 37 C. are very interesting from a physiopathologic point of view. In 1946 Lubinski and Goldbloom[14] could find only 6 cases in the literature, all of which were cases of acquired hemolytic anemia. Knisely *et al.*[11] observed a "sludging" of the blood *in vivo* in shock, for example. This "sludging" is hardly due to an antigen-antibody reaction, and is thus not agglutination in the true serologic meaning.

In our Case 2, auto-agglutination was manifest at 37 C. Clinically there was healing of the lesions of the fingers and toes, and at autopsy there were relatively few signs of sepsis. One, therefore, obtained the impression that the patient's sepsis had been arrested. During life, however, the patient's shock-like condition persisted and hemoglobin was found in the urine. At autopsy, blood pigment in

different organs indicated that the patient had had intravital hemolysis, and a nonseptic thrombus was found in the left internal carotid artery. It seems possible that auto-agglutination played a role in the origin of the thrombus.

The cases observed by us stress the necessity of making a double determination in blood grouping, *i.e.*, both of the serum and of the blood cells. If, in cases such as these, only the blood corpuscles are tested, the patient may be erroneously assigned to blood group AB, and were a blood transfusion to be given, it could have serious consequences.

Auto-agglutination at 37 C. seems to conflict with the serologic rule called "horror autotoxicus", according to which an organism is unable to form antibodies against an antigen already present in the organism in question. There are, however, exceptions to this rule. Thus, for example, blood-group substance A can sometimes be found simultaneously with alpha agglutinins in certain cattle. Hartmann and Friedenreich[10] have recently discussed this phenomenon.

SUMMARY

In 2 patients infected with pyogenic microorganisms, the red blood cells were agglutinated by normal serums independent of alpha and beta agglutinins. In Case 1 the abnormality was temporary and the patient recovered. Case 2 showed auto-agglutination at 37° C. At this temperature the titer of the irregular agglutinins against the patient's own blood cells was considerably higher than against normal cells. The patient expired and at autopsy signs of intravital hemolysis and a thrombus in the left internal carotid artery were found.

The cases are described in the light of similar material published earlier. Case 2 seems to be unique.

REFERENCES

1. Basil-Jones, B., Sanger, R. A., and Walsh, R. J.: Agglutinable factor in red blood cells. Nature, London, **157**: 802, 1946.
2. Benhamou, E., and Nouchy, A.: Grande auto-agglutination des hématies précédée et suivé de grande auto-agglutination des plaquettes. Presse méd., **41**: 25–28, 1933.
3. Boorman, K. E., Loutit, J. F., and Steabben, D. B.: Poly-agglutinable red cells. Nature, London, **158**: 446–447, 1946.
4. Burnet, F. M., McCrea, J. F., and Stone, J. D.: Modification of human red cells by virus action. Brit. J. Exper. Path., **27**: 228–236, 1946.
5. Currie, J. P.: Acute haemolytic anaemia. Brit. M. J., **2**: 8–10, 1944.
6. Davidsohn, I., and Toharsky, B.: Bacteriogenic hemagglutination. J. Immunol., **43**: 213–225, 1942.
7. Forbes, G. B.: Autohemagglutination and Raynaud's phenomenon. Brit. M. J., **1**: 598–600, 1947.
8. Friedenreich, B.: The Thomsen Hemagglutination Phenomenon. Copenhagen: Levin and Munksgaard, 1930.
9. Gaffney, J. C., and Sachs, H.: Polyagglutinability of human red blood cells. J. Path. and Bact., **55**: 489–492, 1943.
10. Hartmann, G., and Friedenreich, V.: Contribution to the discussion on the presence of antigen and the corresponding antibody within the same organism. Acta path. et microbiol. Scandinav., **20**: 45–63, 1943.
11. Knisely, M. H., Eliot, T. S., and Bloch, E. H.: Sludged blood in traumatic shock. Arch. Surg., **51**: 220–236, 1945.
12. Levine, P., and Katzin, E.M.: Temporary agglutinability of red blood cells. Proc. Soc. Exper. Biol. and Med., **30**: 167–169, 1938.
13. Liedholm, K., and Jacobsson, K.: Hemoglobinuria paroxysmalis e frigore non luetica. Nord. med., **24**: 2252, 1944.
14. Lubinski, H., and Goldbloom, A.: Acute hemolytic anemia associated with autoagglutination with a thermal amplitude of 0 to 37 C. Am. J. Dis. Child., **72**: 325–333, 1946.

15. Neber, J., and Dameshek, W.: Improved demonstration of circulating antibodies in hemolytic anemia by the use of a bovine albumin medium. Blood, **2**: 371–380, 1947.
16. Rosenthal, F., and Corten, M.: Über das Phänomen der Autohämagglutination und über die Eigenschaften der Kältehämagglutinine. Folia haemat., **58**: 64–90, 1937.
17. Stats, D., and Bullowa, J. G. M.: Cold hemagglutination with symmetric gangrene of the tips of the extremities; report of case. Arch. Int. Med., **72**: 506–517, 1943.
18. Stats, D., and Wasserman, L. R.: Cold hemagglutination; an interpretive review. Medicine, **22**: 363–424, 1943.
19. Wasastjerna, C.: Acute and subacute hemolytic anemia. Nord. med., **37**: 113–117, 1948.
20. Wiener, A. S.: Conglutination test for Rh sensitization. J. Lab. and Clin. Med., **30**: 662–667, 1945.

CLINICOPATHOLOGIC CONFERENCE*

JACK D. KIRSHBAUM, M.D.†

From the Department of Pathology, Kern General Hospital, Bakersfield, California

CLINICAL DATA

History. The patient was a 59 year old white man who was first seen at this hospital January 26, 1948, having received a deep laceration of the right hand for which he was given first aid. When seen again, February 17, 1948, it was noted that serous fluid was oozing from the wound. He stated that he had lost between 30 and 35 pounds in weight during the past six weeks, his appetite was poor, and he noticed a cough productive of mucoid material. He was unable to walk more than a couple of blocks without stopping to rest. He denied hemoptysis or night sweats and had no pain in his chest, nausea or vomiting. An x-ray film of the chest, taken January 18, showed nothing unusual. It was thought that bronchiectasis was present and he was seen again in the clinic on February 20. At that time the wound on the hand had healed, but he had pitting edema of the left leg distal to the knee and much tenderness of the calf. Homan's sign was positive. It was thought that he had venous thrombosis in the left leg. The patient was advised to return in a week and was given palliative treatment at home. On February 24, he returned to the medical clinic, still complaining of the painful swelling of the lower part of the left leg and ankle and some dyspnea on exertion. His temperature was normal and his pulse rate was 80 per minute. It was noted that his abdomen was pendulous at this time, and free fluid was thought to be present. The liver was easily palpable 5 or 6 fingersbreadth below the right costal margin. There were innumerable ecchymoses over the trunk and legs, but trauma to these areas was denied. Homan's sign was still positive. There was tenderness of the left calf and the left popliteal area. There was generalized excoriation of the skin which was attributed to pediculosis. There was much pitting edema of the left ankle and some tenderness. It was thought at this time that the hepatomegaly might be due to fatty portal cirrhosis. This was associated with thrombophlebitis of the left popliteal vein and with malnutrition. Because of the generalized ecchymoses, the possibility of a blood dyscrasia was also considered. A roentgen film of the chest was considered indicative of arteriosclerotic hypertensive heart disease.

The patient was admitted to the hospital on March 3. He stated that for the past five weeks his appetite had been poor and he had been losing weight. He was unable to explain the loss of weight. In the past, however, the patient had been a derelict and had been a heavy drinker, consuming at least a quart of Arkansas "moonshine" daily for some years until 1943. He denied any drinking since that time. He had not suffered from headaches or sore throats. He stated

* Received for publication, April 8, 1948.

† Present address: Pathology Department, San Bernardino County Hospital, San Bernardino, California.

that while in Arkansas, he had had a "slow fever". He had no operations, but multiple fractures as a result of automobile and train accidents.

Family history. The patient's mother and one sister had died of cancer of the stomach.

Physical examination revealed a fairly well developed but undernourished, unkempt white man. He was dyspneic and had to sit up in bed in order to be comfortable. Examination of the lungs was negative. The cardiac rhythm was regular, and soft aortic and apical systolic murmurs were heard. The blood pressure was 172/104. The abdomen was markedly distended and nontender. A fluid wave and shifting dullness were elicited. A large palpable and movable mass filled the entire right side of the abdomen and extended far below the right costal margin. The rectal examination disclosed no hemorrhoids or masses, and the prostate was small. There was pitting edema over the ankles and numerous ecchymotic areas over the forearms and both legs.

Laboratory findings. Urinalysis: color amber, specific gravity 1.009, albumin and sugar negative, about 40 or 50 leukocytes per high power field; hemoglobin 14.1 Gm. (91 per cent); leukocyte count 9000 with 74 per cent neutrophils, 22 per cent lymphocytes, 2 per cent monocytes, and 2 per cent eosinophils; blood Kahn test, negative; clotting time, 8½ minutes; bleeding time, 5 minutes; prothrombin time, 21.1 seconds (81 per cent of normal, control 17.1 seconds); icterus index, 10.3; total protein, 7.5 Gm.; ascitic fluid, gross blood present, 6 Gm. protein per liter; cephalin-flocculation test, 4 plus at 24 and 48 hours. Electrocardiograms showed evidence of hypertension and coronary sclerosis.

Course. Clinical impression at admission was cirrhosis of the liver with ascites, hypertensive cardiovascular disease with some congestive failure, avitaminosis and possible hypoproteinemia. On March 5, 5 liters of bloody fluid was removed by abdominal paracentesis, affording the patient considerable relief. The fluid contained 6.0 Gm. of protein, per 100 ml. and the sediment showed no tumor cells. Serous fluid continued to ooze from the abdominal wound, the patient became stuporous, and his condition appeared to be getting worse. He complained of aching pain in the right shoulder and chest, but he did not have hemoptysis or cough. An x-ray film of the chest disclosed a suspicious area of density anteriorly at the outer border of the left fifth rib. This area was then found to be present in a previous film, but not in the film taken on February 17. Although the lesion was not considered to be a metastasis, there was destruction of the third rib posteriorly. This led to the impression of primary carcinoma of the lung or possibly plasma cell myeloma. Because of the severe pain, he was given codeine and demerol hydrochloride.

On March 9, a consultant found a mass in the abdomen and ascites, and advised peritoneoscopic examination. On March 10 the patient continued to complain of chest pain and the possibility of pulmonary embolism was entertained. Peritoneoscopy was performed on March 12. The liver was irregular, had the gross appearance of cirrhosis, and in addition there were a few scattered nodular patches suggesting malignancy. Tissue taken for histologic study disclosed tumor cells arranged in acini resembling primary hepatocellular carcinoma, marked periportal cirrhosis, and heavy deposits of hemosiderin.

The patient became weaker, lapsed into coma and expired March 15, 1948, one month after the onset of his acute illness and three months after the appearance of the first symptoms. Postmortem blood chemistry showed nonprotein nitrogen of the blood, 100 mg. per 100 ml.; creatinine, 4.8 mg.; thymol turbidity test, 7 units (normal 1–4 units).

DISCUSSION

Dr. Robert F. Foote, surgeon. "I should like to say a word concerning peritoneoscopy. This method is particularly useful in differential diagnosis of chronic obscure abdominal conditions. Here biopsy may help to establish the diagnosis and permit intelligent treatment. Perhaps the greatest value of peritoneoscopy may be in the study of diseases of the liver, spleen and stomach.

"Cirrhosis of the liver presents a distinctive picture. Suspicion of malignancy of the liver is also an indication for peritoneoscopy. Frequently the clinical differentiation between cirrhosis and neoplasm of the liver is difficult. In addition intraperitoneal hemorrhage, whether it be spontaneous or from a ruptured ectopic pregnancy, may sometimes be determined and in the critically injured patient with possible rupture of a viscus, peritoneoscopy may prevent an unnecessary laparotomy.

"While the examination may easily be carried out, it is not without danger. It should not be carried out in the presence of acute abdominal conditions or intra-abdominal suppurative infection. It is also contraindicated in acute cardiac conditions with decompensation, in view of the possible further reduction in vital capacity secondary to the pneumoperitoneum. Perforation of a viscus during the examination has been reported. Bleeding from the wound may be controlled by coagulative measures. On one occasion intestinal obstruction occurred at the site of insertion of the peritoneoscope three weeks after examination.

"In properly indicated cases, peritoneoscopy has much to offer because of its simplicity and ease of performance."

Dr. Joseph Brackley, internist. "This case of cirrhosis of the liver illustrates the importance of recognizing and treating the disease in its clinically reversible stage. In the early nonspecific, symptomatic stage the situation may be remedied by properly controlling the diet, since malnutrition is important in the genesis of portal cirrhosis. In these instances a high protein, high carbohydrate diet with a moderate fat diet should be prescribed, together with large amounts of vitamins A, B and C. Choline and methionine should be administered to prevent the deposition of large amounts of fat in the liver, and moderation in consumption of alcoholic beverages should be counseled. In acute hepatitis, however, alcohol is contraindicated and a high blood sugar should be maintained in order to pile up protective carbohydrates and proteins in the liver. Long observation and dietary management in these instances are important.

"Attention is called to the symptoms of pectoral alopecia, gynecomastia, and testicular atrophy. Recent work has indicated that the liver in portal cirrhosis is able to inactivate androgen but not theelin or theelin-like substances; part of this failure to inactivate estrogens is believed to be due to the absence of vitamin

B in the liver. In the male the testicular atrophy is probably part and parcel of a malnutritional complex in which the liver is also involved. However, the processes in the two organs may go on at different speeds so that while androgen and estrogen may both decrease, the inability of the liver to inactivate estrogen may outstrip the gradual failure of production of estrogen. As a result pectoral alopecia and gynecomastia may ensue. On occasion, these processes may be reversed by nutritional measures previously noted, *i. e.*, by the use of choline, large amounts of vitamin B complex, parenteral administration of carbohydrates and proteins, and the parenteral use of androgenic substance. The treatment of pectoral alopecia, we believe, is quite discouraging but gynecomastia may be helped."

PATHOLOGIC FINDINGS

Dr. J. D. Kirshbaum. "External examination disclosed pectoral alopecia. The body was in a good state of nutrition and weighed 150 pounds. There was a recent sutured wound 10 mm. long just below the umbilicus. The lower extremities showed no edema.

"In the *abdominal cavity* there was 1000 cc. of a brownish fluid. The lower edge of the liver extended 3 fingersbreadth below the right costal margin. The pleural cavities were free. The *heart* weighed 320 Gm. The coronary arteries were clear except for a few fatty plaques in the intima. The valves appeared normal. In the periphery of the lower lobe of the right *lung* there were two subpleural, firm, light gray nodules measuring up to 15 mm. in diameter.

"The *liver* weighed 3630 Gm. The surface was finely nodular and there were scattered white firm areas measuring up to 15 mm. in diameter. On the anterior surface of the right lobe there was a recent surgical puncture wound. On section the liver appeared dark brown, showed increased fibrosis and indistinct lobulation and scattered firm grayish white tumor nodules. The largest of these was found in the right lobe and measured 70 mm. in diameter. The other nodules averaged about 40 mm. The *gallbladder* appeared normal.

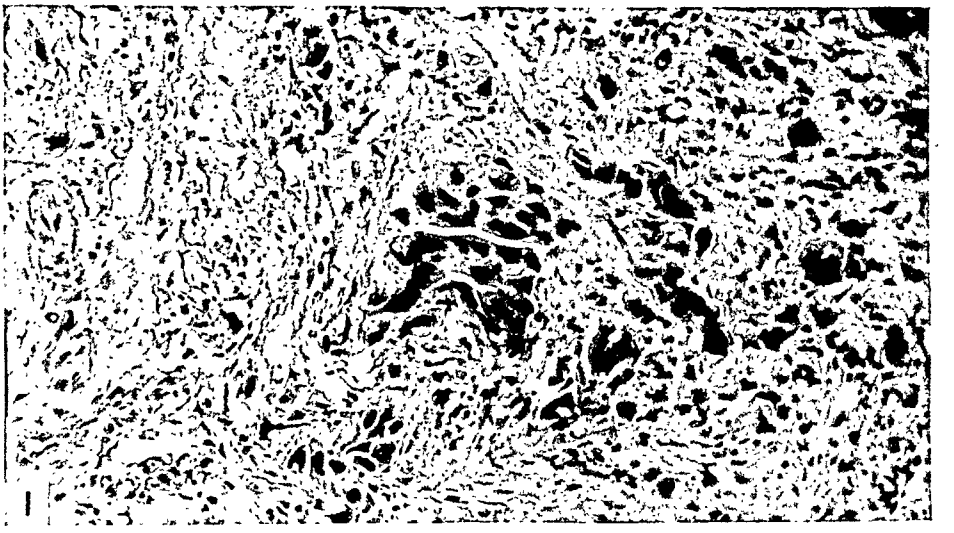
"The *spleen* weighed 340 Gm. It was soft, and the pulp was purple-red and moist with blood. The *pancreas* weighed 160 Gm. On sectioning it appeared dark brown, and the lobules were indistinct. The mucosa of the *stomach* showed marked atrophy. The *kidneys* together weighed 430 Gm. They were of similar size, their consistency was soft, and the capsules stripped with ease exposing dark purplish brown surfaces. On the lateral aspect of the right second, fifth and eighth *ribs* there were nodules of soft, grayish white tumor tissue. These nodules measured up to 35 mm. in diameter. The peripancreatic and periaortic *lymph nodes* were enlarged, matted together and measured up to 25 mm. in diameter. On section they were dark reddish brown."

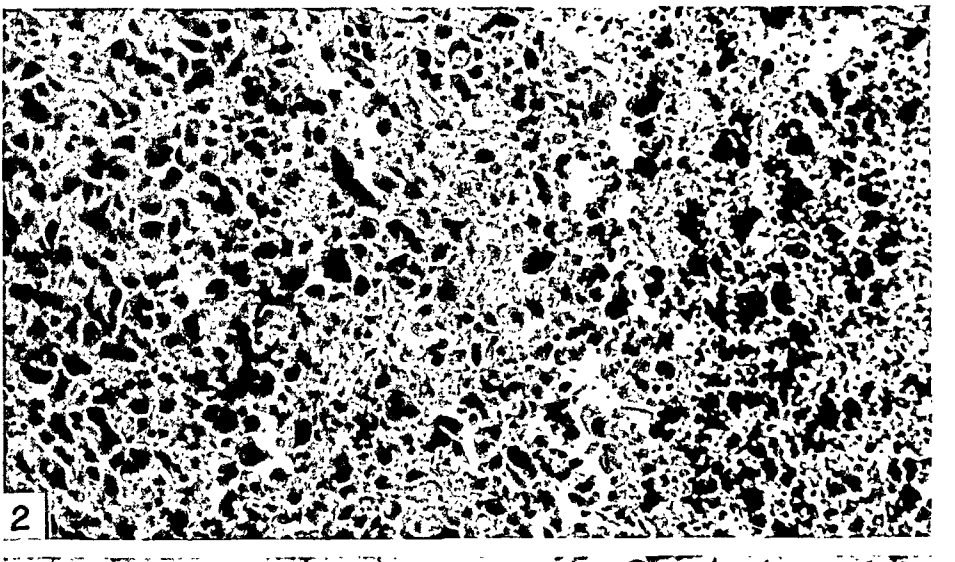
Microscopic examination. Sections of the liver revealed an advanced periportal

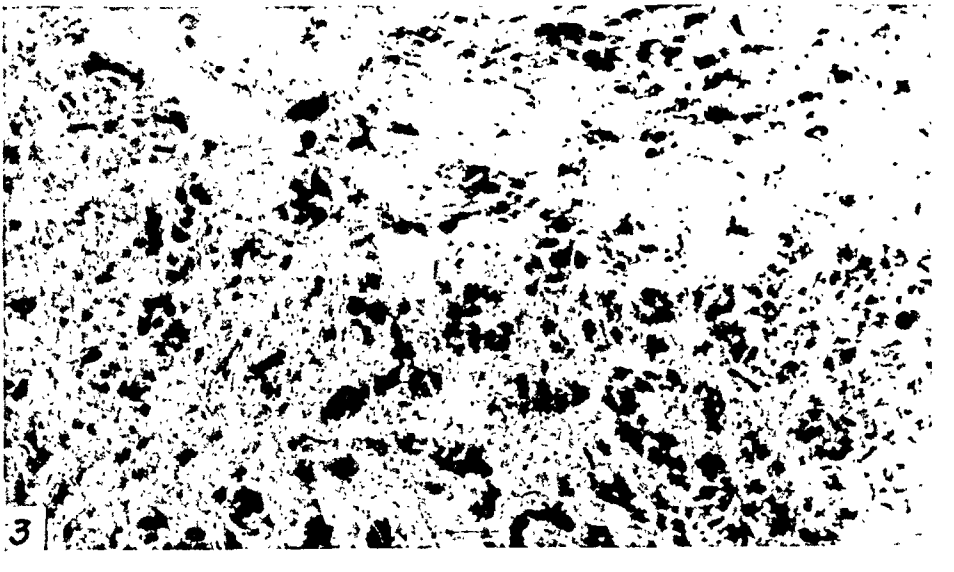
FIG. 1. Groups of tumor cells embedded in a dense fibrillar stroma within the liver.

FIG. 2. Section from liver (H&E) shows carcinoma of liver cell type on left and heavy deposits of iron pigment on right.

FIG. 3. Prussian-blue reaction for iron in liver. Note the heavy deposits of hemosiderin granules typical of hemochromatosis.


1


2


3

cirrhosis with scattered islands of tumor cells (Fig. 1). These cells were arranged like liver cords. Frequently, some of the cells showed multilobulated nuclei. There was extensive fibrosis noted. Large deposits of a reddish brown pigment were present within liver cells and occasionally within some of the tumor cells (Fig. 2). These deposits were also seen in the areas of fibrosis. The pigment gave the Prussian blue reaction for hemosiderin (Fig. 3). Sections of the kidneys showed extensive tubular necrosis. Most of the glomeruli showed large cellular tufts with some fusion of capillaries. Skin sections were negative for iron.

Anatomic diagnoses. 1. Periportal cirrhosis with hemochromatosis of the liver, pancreas, peripancreatic and periaortic lymph nodes. 2. Hepatocellular multicentric carcinoma with metastasis to the right second, fifth and eighth ribs and periaortic lymph nodes. 3. Fibrosis and atrophy of the testes. 4. Focal myocardial fibrosis.

COMMENT

This case illustrates the association of hemochromatosis and primary hepatocellular carcinoma, which is not uncommon. Although there were heavy deposits of iron pigment present in the pancreas, the patient showed no evidences clinically of diabetes. Diabetes was absent in 2 of our last 3 cases of pigmentary cirrhosis.

The bronzed appearance of the skin was also absent. Sections taken from the skin were negative for iron pigment. Various statistics indicate that 25 per cent of cases show no bronzing of the skin, while another 25 per cent show no diabetes.

Pectoral alopecia is a frequent finding in cirrhosis of the liver and its significance as a diagnostic aid is often overlooked clinically. Patients with alcoholic cirrhosis of the liver develop a marked fibrosis and atrophy of the testicles, loss of testicular hormone, frequently pectoral alopecia and a female distribution of pubic hair. Three patients with pigmentary cirrhosis seen lately all showed pectoral alopecia. In a review of 356 fatal cases of alcoholic cirrhosis of the liver, Kirshbaum and Shure[1] noted that 37 per cent of the subjects showed pectoral alopecia at autopsy. Whether alcohol is a contributing factor in the etiology of pigmentary cirrhosis is a moot question. Many of the patients do give a history of heavy drinking. Recently Gillman and Gillman[2] noted hemochromatosis as a sequel of pellagra in 15 per cent of their cases. Malnutrition is an important etiologic factor in cirrhosis of the liver.

In sections of cirrhotic livers studied for iron, the amounts present are usually minimal and, therefore, it is believed that the pigment does not predispose to cirrhosis. However, in pigmentary cirrhosis the reverse is true. Other factors besides alcohol frequently mentioned in the history of patients with hemochromatosis include chronic malaria, syphilis, exposure to copper, previous hepatitis with jaundice and exposure to various toxins and bacterial and chemical agents.

Carcinoma of the liver was present in 2 of our last 3 cases of pigmentary cirrhosis. Ashlag and Martin[3] noted that in 7 per cent of cases of hemochromatosis there was associated primary carcinoma of the liver, and they collected 37 cases from the literature. Their patient gave a history of gonorrhea and syphilis, and

moderate consumption of alcohol. Kirshbaum and Shure[1] noted that in 3 per cent of cases of cirrhosis of the liver, there was associated carcinoma, while in 37 per cent of cases of primary carcinoma of the liver there was a diffuse cirrhosis.

The pathologic diagnosis of hemochromatosis is based on (1) pigmentary cirrhosis of the liver, (2) fibrosis and pigmentation of the pancreas, and (3) siderosis in other organs. Clinically, pigmentary cirrhosis, as in our patient, may not be associated with evidences of diabetes or bronzing of the skin.

REFERENCES

1. KIRSHBAUM, J. D., AND SHURE, N.: Alcoholic cirrhosis of the liver. J. Lab. and Clin. Med., 28: 721–731, 1943.
2. GILLMAN, J., AND GILLMAN, T.: Structure of the liver in pellagra. Arch. Path., 40: 239–263, 1945.
3. ASHLAG, J. A., AND MARTIN, R. F.: Hemochromatosis associated with primary adenocarcinoma of the liver. Am. J. M. Sc., 210: 245–251, 1945.

EDITORIAL

Pathways in Pathology

In the spring of 1877 William Henry Welch wrote his father an enthusiastic letter describing Cohnheim's approach to the study of disease. He compared the newer teachings of Cohnheim with the cellular pathology of Virchow as exemplified by Wagner, Virchow's disciple:

"Prof. Cohnheim and Prof. Wagner are in some respects the antipodes of each other. Wagner has perhaps a greater array of facts at his disposal . . . has gone deeper into the microscopic details of a pathologic change, but while Wagner is often satisfied with the possession of a bare fact, Cohnheim's interest centers on the explanation of the fact. . . . He is almost the founder and certainly the chief representative of the so-called experimental or physiological school of pathology. That is, he occupies himself . . . with the study of the diseased processes induced artificially on animals."[1]

Throughout his long and distinguished career Welch persisted in his efforts to find the answer to the "Why?" of Cohnheim rather than to the "What?" of Virchow, and this single-minded purpose notably changed the direction of pathology in the United States. Welch's influence has been perpetuated and extended by his many associates even into the second generation, profoundly affecting medical thought and practice.

Traditionally, however, pathology is the study of tissue changes resulting from disease and is concerned with cellular and structural alterations. This concept is perpetuated by the American Board of Pathology which grants certificates in "Pathologic Anatomy". Undergraduate teaching of Pathology emphasizes in didactic lectures and laboratory microscopy, the cellular changes observed in growth, repair, inflammation and neoplasia, with supplementary demonstrations of organs and tissues obtained at necropsy and biopsy. Although pioneering teaching-departments of Pathology attempt to correlate clinical and anatomic manifestations, and utilize the Cohnheim principle of experimental pathology, by and large, the major emphasis is on cell structure and morphology.

If this is true with regard to undergraduate teaching, it is certainly true in the training of pathologists. Here the emphasis is on the performance of the autopsy, the study of surgical specimens, and the microscopic analysis of tissue segments. Where investigative activities are included in the course of training, the study of problems involving morphology is as frequently pursued as those requiring animal research. In a few schools the trainee may be required to spend variable periods in the bacteriology laboratory, but usually only as an adjunct to the accumulation of experience in tissue morphology. Few, if any, university training centers have as yet established a rounded program encompassing all of the fields of activity which are of daily importance to the hospital pathologist.

There is growing recognition of the sharp distinction between the hospital pathologist and the university teacher of pathology. In the great majority of instances, the hospital pathologist functions as the only senior medically trained person in his laboratory. He is assisted by one or more interns and residents, and

is responsible for the conduct and interpretation of autopsies, for the diagnosis of surgical specimens, and for supervisory control and standarization of a great variety of technical procedures. These embrace the whole realm of laboratory medicine, and include the disciplines of microbiology, biochemistry, hematology, serology and blood-banking. The determinations are made by technicians of greater or lesser competence (fortunate is the hospital pathologist whose laboratory staff includes a well-trained specialist of Master's or Doctor's grade), but the accuracy of the tests and their interpretation are pre-eminently the responsibility of the pathologist. Aside from his purely localized activities in the laboratory, the hospital pathologist is sought out by his physician-confreres for clinical consultation on diagnostic and therapeutic procedures. Tissue pathology becomes only a relatively minor part of his day's activities, and as the pathologist spreads his influence throughout the hospital in conferences and consultations and committee deliberations, his training in morphologic pathology receives less and less emphasis. The hospital pathologist actively seeks clinical contacts in order both to increase his understanding of disease, and to apply his specialized knowledge in the broad field of laboratory science to the patient's welfare.

These responsibilities of the hospital pathologist differ from the science of pathology as taught in medical schools. Here morphologic pathology is the particular province of the pathologist, and the ancillary disciplines of microbiology, serology, biochemistry are pursued by recognized specialists in these fields. Fundamental concepts of disease processes are stressed, rather than the application of these principles to the care and treatment of the sick patient. Herein lies the crucial paradox, for pathology in the ivory tower of the medical school is concerned with basic principles of disease as indicated by morphologic changes, while pathology as practiced in hospitals is a dynamic activity mainly directed at solving the medical and surgical problems of the living patient.

Surely there is need for reorientation on the place of pathology in the medical curriculum and in the training of pathologists. The preclinical sciences are now presented as a series of unrelated courses, with no great attempt to correlate them into a unity whose major purpose is an understanding of disease. The medical student is expected to weave all of the dangling threads together into a single cloth, and in many instances this effort is unsuccessful. The anatomy of the lung is taught in one part of a year, its histology in another part of the same year, the physiology of respiration at still another time and the pathology of pneumonia a year later; and when all of this information has been tucked away in a corner of his mind, the student is introduced a year or two thereafter to the patient with pulmonary disease, and he learns about diagnosis and treatment. At no time during his medical student days is an attempt made to assist him in integrating all of the specialized information he has acquired. The course in pathology sometimes serves this purpose in limited fashion, for many students have declared that they have learned more anatomy, more histology, more physiology in their year in pathology than they did when actually studying these subjects, but carefully planned and systematic attempts at such correlation would greatly increase the student's comprehension of disease processes.

It is suggested that all of the subjects taught in medical schools be classified in one or the other of two University Departments, the Department of Laboratory Medicine and the Department of Clinical Medicine. The former would include the divisions of anatomy, physiology, pathology, pharmacology, microbiology, biochemistry and medical physics, and the latter would be composed of all of the clinical branches of medicine and surgery. This proposal envisages the abolition of the sharp cleavage currently existing between the so-called preclinical and clinical departments. Courses in the Department of Laboratory Medicine would continue throughout the four years of undergraduate training. Disease would be studied and taught as an integrated whole, and not in separate compartments of unrelated information. In this program, pathology, the study of disease rather than the study of the tissue changes produced by disease, would assume its dominant role, for instead of being presented only in the second year, it would help to revitalize the teaching program of the clinical years.

It is not the purpose of this program to interfere with present methods of teaching the preclinical branches of medicine. Lectures, demonstrations and laboratory exercises would continue as at present, in perhaps slightly modified form in order to accommodate a new course. This would be presented on a continuing basis throughout the four years of medical school. It would be an integrating, consolidating, correlating course, given by members of the Department of Laboratory Medicine. To paraphrase Pope, its purpose could be stated thus: "The proper study of disease is disease itself". It would be presented by a panel of scientists representing all of the preclinical fields. The topical outline would be an expansion of the currently taught subject of systemic pathology. In a discussion of pulmonary diseases, the anatomist would first review the anatomy of the lungs and the thoracic cage and diaphragm, the histologist would present the histology of pulmonary tissue, the physiologist would discuss the physiology of respiration, and the chemist the chemistry of gaseous exchange, while the bacteriologist would present the newer knowledge of etiologic agents in pulmonary disease, and the pathologist the tissue alterations noted on gross and microscopic examination. The course envisaged would be patterned after the very successful survey courses given in some undergraduate colleges, that in Contemporary Civilization at Columbia for example, and its direction and over-all supervision would be controlled by the pathologist.

And what of the clinical years? Here too, the Department of Laboratory Medicine would be active. Staff members representing the preclinical branches would be attached to the divisions of medicine and surgery, and their specialized knowledge would be available on ward rounds and teaching seminars. The physiologist, the anatomist, the chemist, the bacteriologist and the pathologist should all be available for deliberation and discussion at the patient's bedside. And the pathologist in particular should leave the autopsy room for daily consultation on the ward. This attempt to integrate the basic sciences into the clinical experience of the undergraduate student will impart a point of view and an approach to disease which should prove of immeasurable value in his clinical practice as a graduate physician.

The Department of Laboratory Medicine not only would influence the whole pattern of undergraduate medical education, but it would also supervise the postgraduate training of those seeking to specialize in the field of pathology. University centers now train tissue pathologists, many of whom continue in academic careers. However, the number of senior posts in medical school pathology departments is limited, and for this reason the great majority of pathologists sooner or later accept hospital opportunities. They then find themselves in the anomalous position of being experts in tissue diagnosis, a field which constitutes only a small area of their hospital activities, and inadequately trained in the other laboratory sciences which demand most of their attention. This one-sided emphasis would be eliminated by placing the training of pathologists under the direction of the Department of Laboratory Medicine. A four or five year program is suggested, preceded by a year of clinical interneship. The trainee would center his activities in the department of pathology where at all times he would participate in the departmental conferences and demonstrations, and have the opportunity to review gross and microscopic material with the group or alone. He would rotate with others in the conduct of autopsies and in the examination of surgical specimens. At least two years of his period in residence would be devoted to the other specialties in the Department of Laboratory Medicine. In biochemistry and bacteriology, he would be supervised by the departmental chiefs. He would be actively engaged in the performance of the specialized laboratory technics, and participate in the seminars of the Department of Laboratory Medicine, and in ward rounds. He would work with students and supervisors in clinical microscopy, and especially in hematology, and would observe and perform blood bank technics. Special assignments in the departments of physiology and medical physics, in serology, immunology and toxicology would emphasize the value of these disciplines in the understanding of disease processes, and in diagnosis and prognosis. Major stress would be placed on the application of knowledge and technics to the care of the sick. And in addition to these special disciplines, the trainee would be assigned to the administrator of the hospital for instruction in business policies, personnel relations, purchasing and charges. The investigative approach to problems in medicine would receive especial attention, and a carefully planned research project would be pursued by each trainee. On the successful completion of this training period, the young pathologist would with confidence be prepared to accept a hospital position and creditably to perform his duties immediately on appointment.

The responsibility of bringing pathology and the pathologist to the bedside rests not only with university teaching centers. Hospital planners, architects and administrators should also give this problem serious attention. Too often are laboratories of pathology located in remote areas within the hospital, instead of being centralized and in proximity to the clinical wards. The purely technical procedures performed in the laboratory should be made readily available, and this can be accomplished by planning for free and easy reciprocal access between ward and laboratory. This close physical relationship would also stimulate the

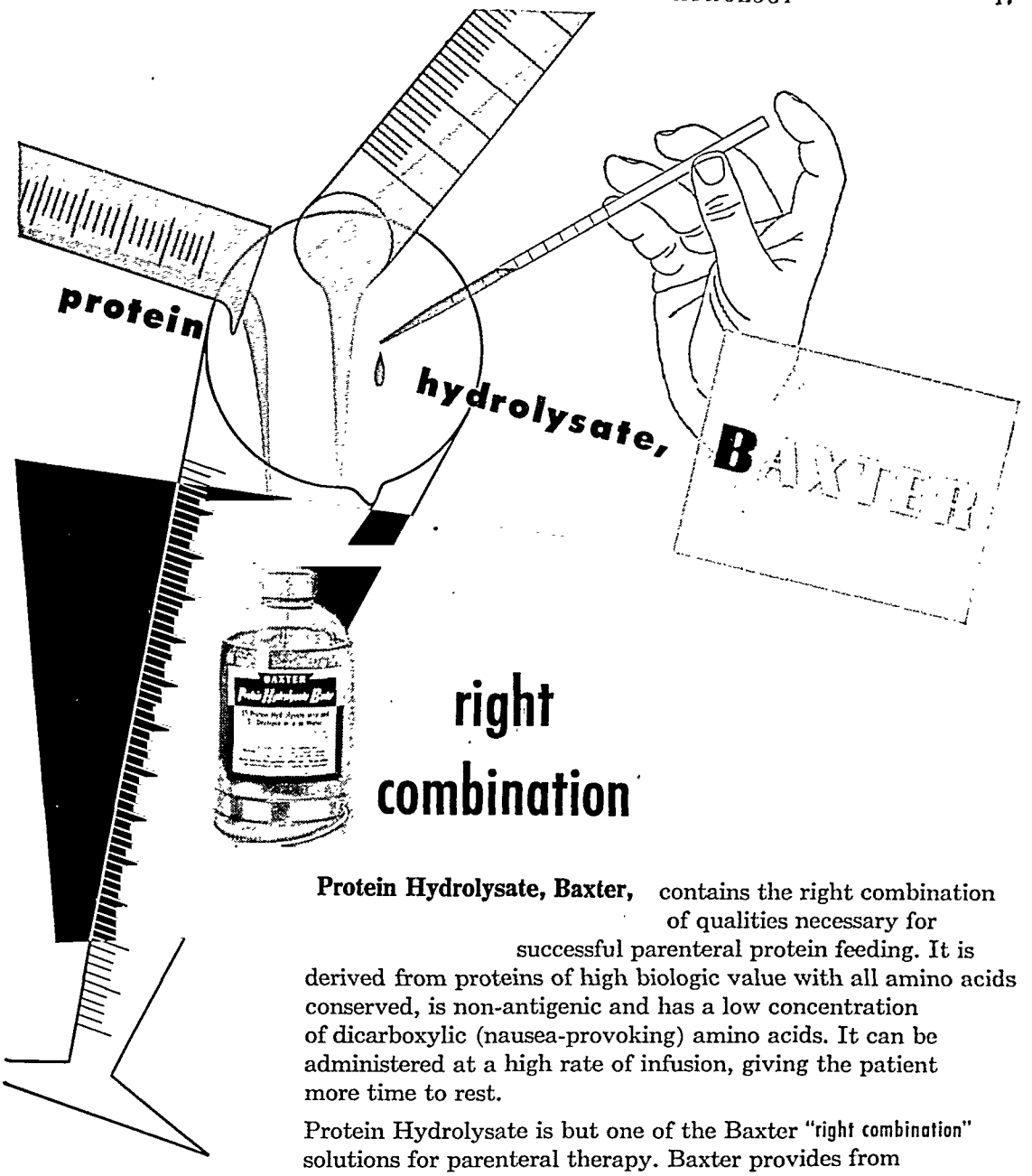
clinical staff to make greater use of the consultative experience of the pathologist, who could quickly and conveniently visit the ward when his advice is desired.

The adoption of the suggestions herein outlined will offer many difficulties, especially to institutions already burdened with fixed traditions and an immovable physical plant. But the role of pathology in clinical medicine has changed considerably since Welch's time. The increasing clinical importance of pathology should receive its proper recognition in the planning of new curricula and new hospital constructions.

New Britain General Hospital PAUL D. ROSAHN
New Britain, Connecticut

REFERENCE

1. FLEXNER, S., AND FLEXNER, J. T.: William Henry Welch and the Heroic Age of American Medicine. Ed. 1. New York: The Viking Press, pp. 94–95, 1941.



right combination

Protein Hydrolysate, Baxter, contains the right combination of qualities necessary for successful parenteral protein feeding. It is derived from proteins of high biologic value with all amino acids conserved, is non-antigenic and has a low concentration of dicarboxylic (nausea-provoking) amino acids. It can be administered at a high rate of infusion, giving the patient more time to rest.

Protein Hydrolysate is but one of the Baxter "right combination" solutions for parenteral therapy. Baxter provides from one source and with standardized procedures the exact solution and the specific equipment for any parenteral requirement.

Product of BAXTER LABORATORIES
Morton Grove, Illinois • Acton, Ontario

BAXTER pioneer name in parenteral therapy

Available only in the 37 states east of the Rockies (except El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION

GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

for

BLOOD TYPING
RH TESTING

you need the highest-titred serum available.

ANTI-HUMAN PRECIPITIN SERUM (rabbit)
for the Coombs Test

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human Red Cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic Serum
for control tests

P.S.A.
(Paraffin Section Adhesive)

Write for complete information.

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York



(In writing to advertisers, please mention the Journal—it helps.)

ANNOUNCING PUBLICATION OF

Handbook of

DIGESTIVE DISEASES

By the late JOHN L. KANTOR, M.D., F.A.C.P., *Associate in Medicine, Columbia University; Gastroenterologist and Associate Roentgenologist, Montefiore Hospital, New York;*
and
ANTHONY M. KASICH, M.D., F.A.C.P., *Lecturer in Medicine, Columbia University; Adjunct Physician, Montefiore Hospital; Assistant Visiting Physician, Bellevue Hospital; Assistant Adjunct Gastroenterologist, Lenox Hill Hospital, New York*

SECOND EDITION

658 Pages 148 Illustrations 2 in Color PRICE, $11.00

A COMPLETE TREATISE ON GASTROENTEROLOGY PRESENTED IN THE MOST COMPACT, READABLE AND PRACTICAL MANNER. ENTIRELY UP-TO-THE-MINUTE IN COVERAGE. CONTAINS A CRITICAL EVALUATION OF THE MUCH DISCUSSED OPERATION, VAGOTOMY.

Handbook of Digestive Diseases is an outgrowth of Dr. Kantor's Synopsis of DIGESTIVE DISEASES, the revision of which was interrupted by his death. In taking up the task of completing the work, Dr. Kasich has enlarged it immensely and rewritten it entirely. This Second Edition presents, first, a concise picture of the more vital and fundamental aspects of digestive diseases; second, the approach is on a sound physiological basis. The fact that gastroenterology is an important and inseperable segment of internal medicine has been stressed throughout.

QUICK FACTS ABOUT ITS COVERAGE

• The clinical resources of Montefiore Hospital, a great teaching institution, have been fully utilized. Thus, the important section on cancer of the pancreas is based upon a study of more than one hundred cases of this disease. The section on ulcerative colitis has been compiled from a study of 143 cases seen at Montefiore. Most of the significant statistical material has been obtained from records at Montefiore.

• X-ray pictures, which are so necessary to the study of digestive diseases, are plentiful and of excellent quality. They have been beautifully reproduced. In many cases, the x-ray is accompanied by an artist's drawing, which calls attention to the salient features.

• The two chapters on peptic ulcer, with the excellent illustrations, form a small monograph on the subject. The modern concepts of the causation of ulcer are thoroughly discussed, in simple language.

• Psychosomatic aspects are stressed, and the symptoms are carefully related to the physiological disturbances which cause them.

• In the discussion of pain in ulcer, the latest researches on the relation of acid secretion are fully described, and the significance of these findings in the management of peptic ulcer are explained. Treatment of ulcer and its complications, in all aspects, is thoroughly covered.

ORDER FORM

THE C. V. MOSBY COMPANY
3207 Washington Blvd.
St. Louis 3, Missouri

JCP 8-49

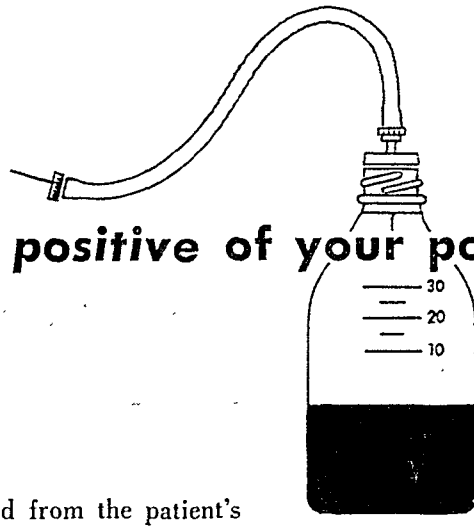
Please send me Kantor-Kasich's Handbook of DIGESTIVE DISEASES—The price is $11.00

☐ Enclosed find check. ☐ Charge my account.

Name

Address

NOW



you can be positive of your positives

Direct delivery of blood from the patient's vein, through a closed, sterilized system, into Trypticase Soy Broth media in the new B-D VACUTAINER CULTURE BOTTLE . . .

- **Provides positive positives and valid negatives**
- **Prevents outside contamination**
- **Avoids incorrect selection and multiplicity of media**

Aerobic or anaerobic conditions may be obtained at time blood specimen is taken. Bottle reaches laboratory ready for immediate incubation . . . speeds laboratory handling . . . provides faster diagnosis. Microaerophiles grow luxuriantly.

Write for descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE and learn how easy it is to add this new element of assurance to your blood culture procedures.

with the

B-D

VACUTAINER®

CULTURE

BOTTLE

containing Trypticase Soy Broth with CO_2 added

Prepared and sterilized by Baltimore Biological Laboratories, Inc., Baltimore, Md.

BECTON, DICKINSON & CO., Dept. 27-II
15 Ames Avenue,
Rutherford, N. J.

Please send me descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE that takes the guesswork out of blood culture procedures.

NAME_____

ADDRESS_____

CITY_____ ZONE____ STATE_____

BECTON, DICKINSON & CO., RUTHERFORD, N. J.

(In writing to advertisers, please mention the Journal—it helps.)

AT HOME OR AWAY SPOT TESTS SIMPLIFY URINALYSIS

No Test Tubes • No Measuring • No Boiling

Diabetics welcome "Spot Tests", (ready to use dry reagents), because of the ease and simplicity in using. No test tubes, no boiling, no measuring; just a little powder, a little urine—color reaction occurs at once if sugar or acetone is present.

Galatest . . . Acetone Test (DENCO)

FOR DETECTION OF SUGAR IN THE URINE — FOR DETECTION OF ACETONE IN THE URINE

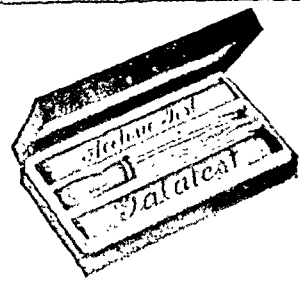
SAME SIMPLE TECHNIQUE FOR BOTH

1. A LITTLE POWDER  2. A LITTLE URINE

COLOR REACTION IMMEDIATELY

Accepted for advertising in the Journal of the A.M.A.

WRITE FOR DESCRIPTIVE LITERATURE

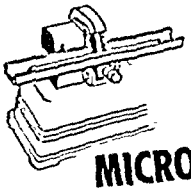


A carrying case containing one vial of Acetone Test (Denco), one vial of Galatest, medicine dropper and Galatest color chart is now available at all prescription pharmacies and surgical supply houses. This is very convenient for the medical bag or for the diabetic patient.

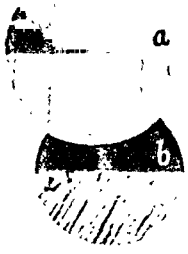
Acetone Test (DENCO) *Galatest*

THE DENVER CHEMICAL MANUFACTURING COMPANY, INC.
163 Varick St., New York 13, N.Y.

c



the Berkeley
MICROTOME KNIFE SHARPENER
assures an
INCOMPARABLE EDGE
3 times faster



COMPARE THE EDGES. Photomicrograph *(a)* shows a microtome knife edge sharpened by the Berkeley sharpener. Note its even smoothness, assurance of better sections. Photomicrograph *(b)* shows a blade laboriously hand sharpened, honed and stropped, but note the nicks and serrations still remaining.

COMPARE THE TIME. In tests, wherever made, and in practice in hundreds of laboratories, the Berkeley Microtome Knife Sharpener gives perfect edges in one-third the time required for hand honing and stropping.

Let us send you Bulletin MKS-14 giving full information about this indispensable laboratory aid.

Berkeley Scientific Company
SIXTH AND NEVIN AVE • RICHMOND, CALIFORNIA

Complete information in one volume

BLOOD DERIVATIVES AND SUBSTITUTES

by Charles Stanley White, M.D.
and Jacob Joseph Weinstein, M.D.

A comprehensive study of plasma and plasma substitutes and by-products with the chief emphasis on practical and clinical aspects.

Covers
Preparation, storage and administration
Indications for use
Shock and its treatment
Use of dried and frozen plasma
Plasma fractions
Organization of blood plasma bank
Transfusions

530 pp., 196 figs., $7.50

The Williams & Wilkins Co.
Mt. Royal & Guilford Aves., Baltimore 2, Md.

(In writing to advertisers, please mention the Journal—it helps.)

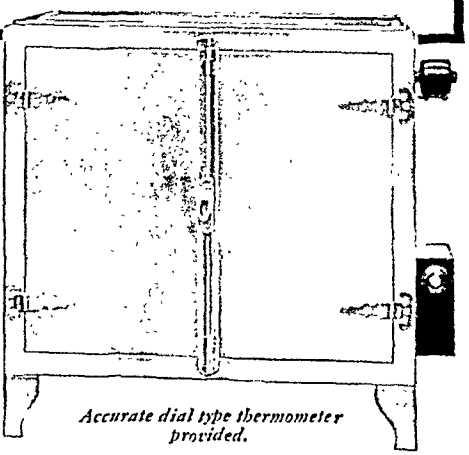
Castle Hot Air Sterilizer

LIFETIME STAINLESS STEEL . . . FOR CONSTANT, EXTRA HEAVY WORK IN THE LABORATORY

The Castle No. 3 Hot Air Sterilizer, by reason of its insulated *and* stainless steel construction, gives longer service, and more accuracy of temperature control.

Circulating fan, powered by heat-resisting motor on outside wall, provides full circulation of air . . . eliminates "hot" and "cold" spots . . . insures uniformity of 4°C.

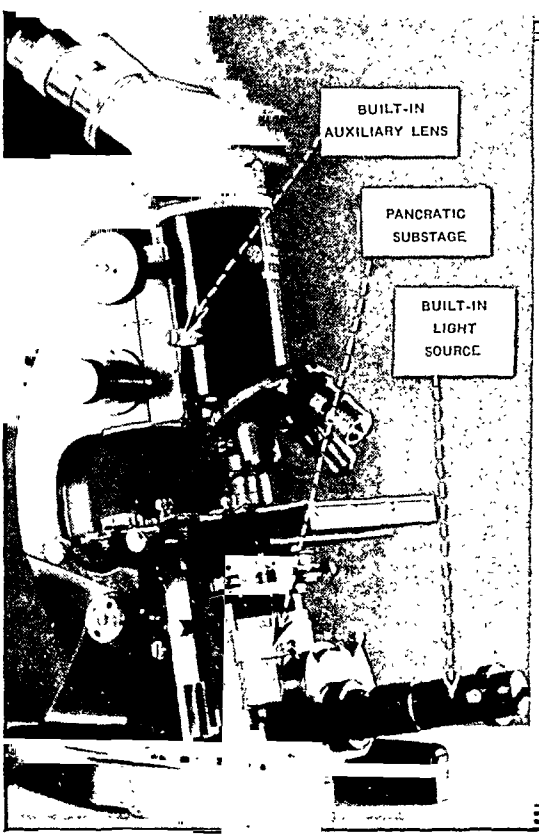
Guaranteed electrical heating units inside sterilizer, with automatic control, hold temperature at *any* point from 38° to 260°C. Exceptional accuracy ot control enables Sterilizer to be used also as a Drying Oven.



Accurate dial type thermometer provided.

For full details, write: Wilmot Castle Company, 1216 University Ave., Rochester 7, N. Y.

Castle BACTERIOLOGICAL APPARATUS



GALILEO CORP. OF AMERICA, Inc.

Announces

The VNP Phase-Contrast Microscope with features which open completely new possibilities in phase-contrast technique.

•

For descriptive brochure write

PFALTZ & BAUER, Inc.

Empire State Building, New York 1, N. Y.

Sole agents for

GALILEO CORPORATION OF AMERICA, Inc.

745 Fifth Avenue, New York 22, N. Y.

Distributor for Officine Galileo, Italy

Florence • Milan • Venice

Since 1881 manufacturers of research microscopes and microscopical equipment of all kinds, refractometers, polarimeters, and analytical balances.

A.M.A. urges increased recruitment for technical training school!

In the "Current Comment" section of the May 7, 1949, issue of the Journal of the American Medical Association, the statement is made: "The student recruitment program must be further intensified with increased counseling at high school and college levels."

Now is the time to make plans to confer with all high schools and colleges in your area.

Give them informational material regarding requirements for certification in Medical Technology.

Offer to counsel students at Career Conferences.

Urge them to consider only the best kind of training.

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
American Society of Clinical Pathologists
Muncie, Indiana

WHY YOU SHOULD SPECIFY

"VACSEAL"

GUINEA PIG COMPLEMENT

Because it is a superior complement—the result of a decade of experience in guinea pig complement production, the proper choice and preparation of animals and the use of improved technics.

Because our large output enables us to pool the serum of no less than 100 guinea pigs per lot, assuring a more uniform titer. It also makes it possible for you to obtain a complete lot of any complement which has proved, through pretesting, to be particularly desirable for your purpose.

Because of the high vacuum and all glass containers, storage for at least two years is possible. To you this means economy and convenience.

"Vacseal" Complement is distributed in three sizes with a special diluent for restoration to the following amounts:

3 ml. 7 ml. 20 ml.

Any recognized laboratory will receive a trial 3 ml. sample upon request.

CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK



XENOPUS LAEVIS FROG

Pregnancy Diagnosis

Animal Costs Greatly Reduced

We assure you of a continuous supply. Accurate, speedy, and inexpensive. Average animal cost per test, 20 cents to 35 cents. We carry a complete stock of Laboratory Tanks, Isolation Jars, Holders, and correct reagents and apparatus for running tests Speedy delivery via AIR EXPRESS. Current brochure showing new low prices sent upon request.

Jay E. Cook, Importer

P. O. Box 302 Baltimore 3, Md., U.S.A.
Cable Address—XENOPUS-BALTIMORE

(In writing to advertisers, please mention the Journal—it helps.)

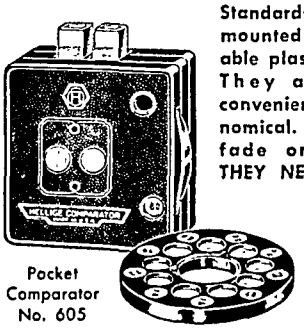
HELLIGE COMPARATORS

FOR BETTER

pH Control

there is nothing like

NON-FADING GLASS COLOR STANDARDS



Pocket Comparator No. 605

Hellige Glass Color Standards are safely mounted in unbreakable plastic frames. They are more convenient and economical. They never fade or change, THEY NEVER FAIL.

WRITE FOR BULLETIN No. 602

HELLIGE

INCORPORATED

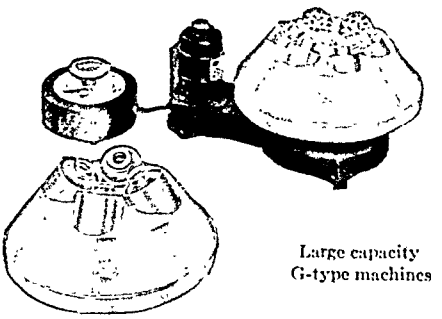
3718 NORTHERN BLVD., LONG ISLAND CITY 1, N.Y.

HEADQUARTERS FOR COLORIMETRIC APPARATUS

SERVALL

ANGLE CENTRIFUGES

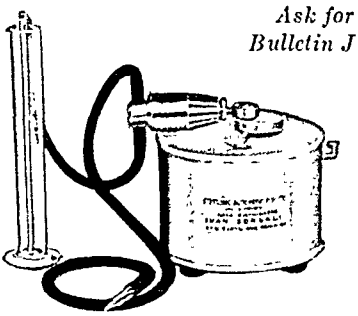
Ask for Bulletin J-81 with complete data on all models



Large capacity G-type machines

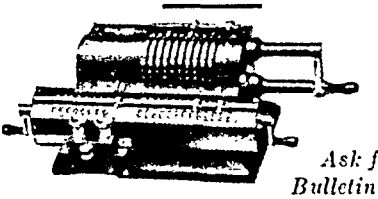
Type G/1, for 6 bottles of 250 cc each
18 tubes of 60 cc each
42 tubes of 15 cc each
78 tubes of 10 cc each
Type G/2, for 4 bottles of 500 cc each
Type G/3, for 10 tubes of 100 cc each

Ask for Bulletin J-82



STERLING AUTOMATIC AND HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc. Easy to operate—Time-Saving



Ask for Bulletin J-83

ORIGINAL—ODHNER

Portable Calculator for the Scientist
Many different models
Efficient - Sturdy - Low Priced

Manufacturers and Distributors:

IVAN SORVALL, Inc.

210 FIFTH AVE.-NEW YORK 10, N. Y.

Advertisers in the Journal Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.

Labreco

Prepared Clinical Laboratory Reagents for

BIOCHEMICAL
BACTERIOLOGICAL
HISTOLOGICAL
SEROLOGICAL
HEMATOLOGICAL
PROCEDURES

Prepared Bacteriological Culture Media in Sterile Screw Cap Test Tubes—
Petragnani T.B. Medium
One Dozen 2.75
Loeffler's Blood Serum
One Dozen 2.25

HEMATOLOGICAL AND BACTERIOLOGICAL STAINS

CATALOG UPON REQUEST

LABORATORY REAGENTS COMPANY
Woodbridge, New Jersey

World-Wide Abstract Service

EXCERPTA MEDICA

An International Medical Abstract Service published in English and covering the whole field of Clinical and Experimental Medicine

I. Anatomy, Anthropology, Embryology and Histology	$22.50
II. Physiology, Biochemistry and Pharmacology	45.00
III. Endocrinology	15.00
IV. Hygiene and Medical Microbiology	22.50
V. General Pathology and Pathological Anatomy	37.50
VI. Internal Medicine	37.50
VII. Pediatrics	15.00
VIII. Neurology and Psychiatry	22.50
IX. Surgery	25.00
X. Obstetrics and Gynecology	15.00
XI. Oto-rhino-laryngology	15.00
XII. Ophthalmology	15.00
XIII. Dermatology and Venereology	25.00
XIV. Radiology	15.00
XV. Tuberculosis	15.00

Special rate to libraries ordering all 15 sections $250.00

Published monthly, one volume a year. Subscription by volume only, payable in advance. Send for a sample copy of the section which covers your specialty

THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves.
Baltimore 2, Md.

Sole distributor for the United States, Canada and Central America

(In writing to advertisers, please mention the Journal—it helps.)

Media for Enteric Pathogens

for their Isolation and Differentiation

SELENITE-F ENRICHMENT
DESOXYCHOLATE AGAR
D. C. L. S. AGAR
T. S. I. AGAR
TRYPTICASE AGAR BASE
UREASE TEST MEDIUM
PHENOL RED BROTH

Literature and prices sent on request.

BALTIMORE BIOLOGICAL LABORATORY

1640 Gorsuch Avenue Baltimore 18, Md.

Reagents for Determining Prothrombin Time

Thromboplastin Extract Activity Chart Available upon Request

THROMBOPLASTIN
(Dehydrated Rabbit Brain)
6 ampuls (0.15 gram each)..... $4.50

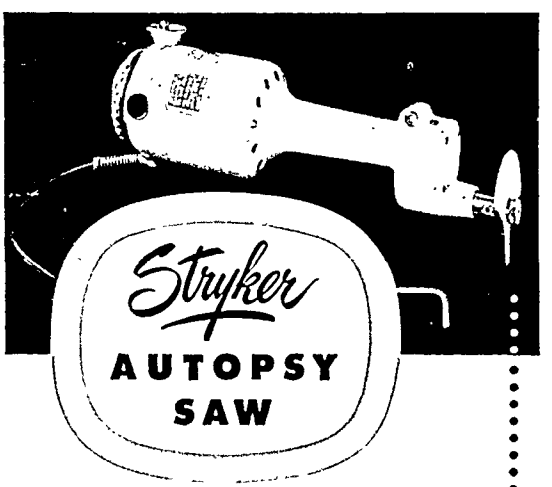
THROMBOPLASTIN EXTRACT
(Desiccated)
1 c.c. ampul..... $1.00
6 ampuls (0.5 c.c. each)..... $4.00
10 individual test ampuls..... ... $2.00

SODIUM OXALATE SOLUTION
(0.1 Molar)
4 oz..... ... $0.75

CALCIUM CHLORIDE SOLUTION (0.02 Molar)
4 oz..... $0.75

NORMAL PLASMA FOR CONTROL (Desiccated)
1 c.c..... $1.00
6 ampuls (0.5 c.c. each)..... $4.00

CAPPEL LABORATORIES
Diagnostic Reagents
Box 352 Wayne, Pa.

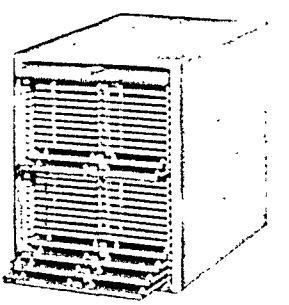


Stryker AUTOPSY SAW

A new instrument which simplifies bone cutting

Electrically driven, oscillates at high speed to cut bone efficiently with complete safety. Cutting blades do not hurl material. Two-sided blade can be adjusted to three positions. Blade, arbor and shaft are stainless steel. Write Department C for complete information.

ORTHOPEDIC FRAME COMPANY Kalamazoo Michigan



EBERBACH UNIT SYSTEM MICRO SLIDE CABINETS

You can have an "expandable" filing system with the slides easy to locate. Each cabinet holds 500 slides flat in individual compartments. Hardwood cabinets 8½ x 10 x 12" deep have flat slides and disappearing door so as to stack neatly. #60-660 cabinet shown above sells for $36.00. Write for Bulletin 150.

Eberbach & Son Company
Ann Arbor, Mich.
Laboratory Apparatus & Supplies

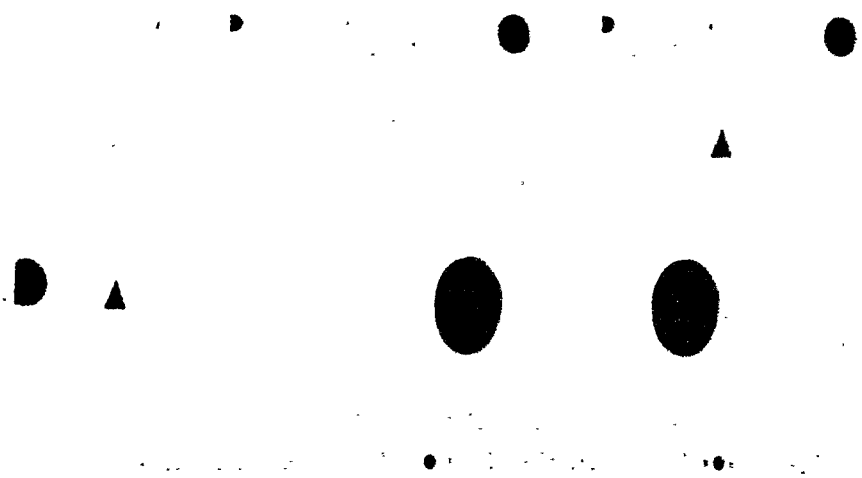
(In writing to advertisers, please mention the Journal—it helps.)

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia...O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2513 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Benbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

VOLUME 19 JULY 1949 NUMBER 7



S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R. BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly [illegible] with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Agents: For the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Australia: Angus and Robertson, Limited, 89-95 Castlereagh Street, Sydney; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 JULY 1949 NUMBER 7

CONTENTS

Medicolegal Examination of Bodies Recovered from Burned Buildings. F. R. DUTRA..... 599
Studies on Inclusion Bodies. I. Acid-Fastness of Nuclear Inclusion Bodies That Are Induced by Ingestion of Lead and Bismuth. M. WACHSTEIN..... 608
Lecithinase Activity in Splenic Dyscrasias. G. J. SCHEFF AND A. J. AWNY..... 615
Studies on the Blood Factor rh^{w}. A. S. WIENER AND E. B. GORDON..... 621
Case Reports
Acute Hemolytic Anemia Associated with Elevated Titer of Cold Agglutinins. I. L. RUBIN, A. S. JACOBSON AND L. M. MEYER..... 630
Massive Stilbamidine Therapy of Multiple Myeloma. T. A. HAEDICKE AND E. M. GREENSPAN..... 634
Leiomyoma Angiomatosum of the Mesentery. PH. H. HARTZ AND F. R VAN DE STADT 639
Clinicopathologic Conference. J. MCGUIRE AND P. N. JOLLY..... 643
Editorial: The Training of Clinical Pathologists. O. A. BRINES..... 653
Selected Abstracts..... 654
Book Reviews..... 655
News and Notices..... 658
Technical Section
Rapid Measurement of Serum Total Base and Estimation of Serum Sodium. An Improved Conductivity Assembly. F. W. SUNDERMAN..... 659
Rapid Macroscopic Test for Infectious Mononucleosis. A Method for Preservation of Sheep Cells. F. RAPPAPORT AND M. SKARITON..... 665
Excretion of Silver in a Patient with Argyrosis. Ineffectiveness of Treatment with BAL. F. S. GERBASI AND A. R. ROBINSON..... 668
Photographic Recording of Hemolysis Using a Portable Electrocardiograph. W. E. LOVE, R. EDELBERG AND E. F. MACNICHOL..... 676
Texchrome Silhouette Staining of Platelets. Morphology *in Vitro* Before and During Coagulation. L. VROMAN..... 681
Culture of Tubercle Bacilli from Specimens of Gastric Juice. P. KABLER AND R. LUNDHOLM..... 685
Use of Clorox and Trisodium Phosphate in Demonstration of Acid-Fast Bacilli in Sputum. M. S. TARSHIS AND W. G. LEWIS..... 688
An Accurate Pipetting Instrument for Serologic Use. A. SVEDMYR..... 693
The Use of Silicone-Coated Needles in Blood Donor Sets. T. J. GREENWALT..... 695
An Improved Blood Culture Bottle Utilizing a Solid-and-Liquid Medium. R. M. MARWIN..... 697
The Determination of Bromsulphalein in the Presence of Hemolysis, Turbidity and Bile. A. FREY AND M. FREY..... 699
A Source of Error in Bromsulphalein Tests. P. JERNSTROM AND E. PERKINS..... 700

(In writing to advertisers, please mention the Journal—it helps.)

THE PANRAY CORP.

pioneer manufacturer of DOPA

and other specialized chemicals

for investigation and research . . .

announces the availability of

Iodoacetic Acid "Panray"

(twice recrystallized, special reagent grade)

for the Iodoacetate Index Determination of Serum Proteins

Iodoacetic acid is a recently reported reagent for the determination of *deficient thermal coagulation of serum proteins* in cancer and other pathological conditions.

Originally reported by C. Huggins, M.D., G. M. Miller, M.D., and E. V. Jensen, Ph.D.: Thermal Coagulation of Serum Proteins—II. Deficient Coagulation in Cancer and the Iodoacetate Index —*Cancer Research, Vol. 9, No. 3, March 1949.*

DOPA "PANRAY"

(3,4-dihydroxyphenylalanine, C. P.)

available in l and dl forms

Synthetic chemical reagents . . . capable of revealing the presence of an oxidase in cells elaborating melanin. Valuable indicators of active melanogenesis.

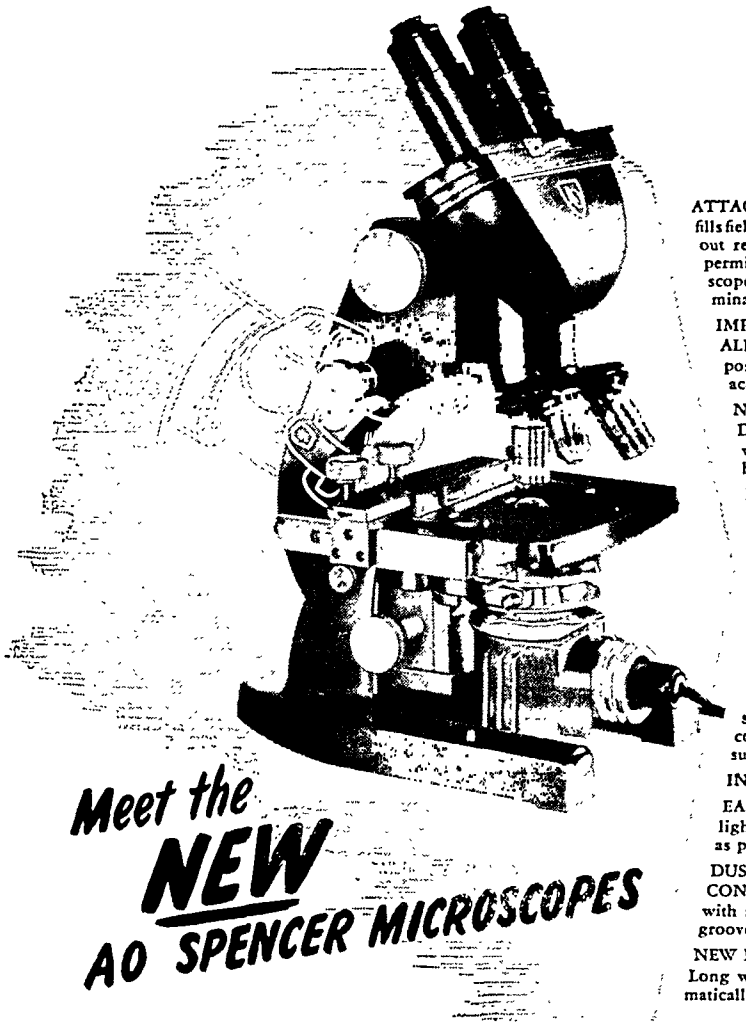
Write for prices and other data

THE PANRAY CORP.

Custom Manufacturers of Fine Organic Chemicals

398 Broadway New York 13, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)



Meet the NEW AO SPENCER MICROSCOPES

ATTACHED ILLUMINATOR
fills field at various powers without refocusing condenser and permits inclination of microscope without disturbing illumination.

IMPROVED SUBSTAGE ALIGNMENT
positive locking device assures accurate optical alignment.

NEW, N.A. .25, 10X DIVISIBLE OBJECTIVE
with cut-away boot—permits better oblique top lighting.

NEW FINE ADJUSTMENT
ball-bearing, micrometer screw type—accurate 2 micron graduations—comfortable lowered position.

"PINCH GRIP" SLIDE HOLDER
provides for inserting slides easily without disturbing stage settings.

ADJUSTABLE TENSION
substage and coarse adjustment controls may be adjusted to suit individual preference.

INTERCHANGEABLE BODIES

EASY TO CARRY
lighter weight—twice as rigid as previous models.

DUST SHEDDING DUAL-CONE NOSEPIECE
with new *positive* ball-and-groove stop.

NEW INCLINATION JOINT
Long wearing—bearings automatically compensate for wear.

NEW features, extra user convenience, and excellent optical performance distinguish every model in this complete new line of laboratory microscopes. See them at your AO Spencer Distributor and ask for literature.

American Optical Company
Scientific Instrument Division
Buffalo 15, New York

Makers of Microscopes for over 100 Years

AO SPENCER

UNEQUALED

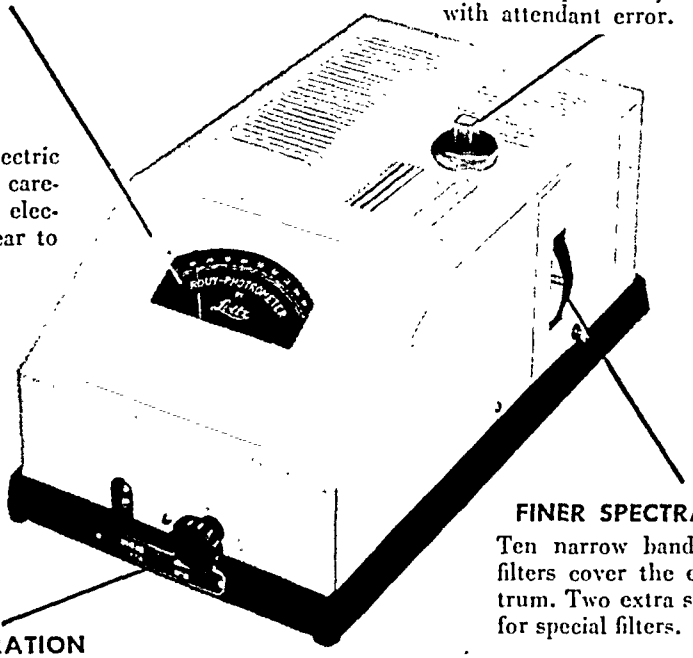
for accuracy · simplicity · dependability

the ROUY PHOTROMETER by Leitz

MORE ACCURATE READINGS
Only the Photrometer cuts functional error to within 0.1%.

GREATER OPTICAL ACCURACY
Precision square absorption cells with plano-parallel surfaces eliminate errors possible with ordinary curved cells. Optical system is designed to eliminate possibility of stray light with attendant error.

BETTER MATCHING OF ELEMENTS
Only Leitz matches photoelectric cell and microammeter so carefully that measurement of electrical energy is strictly linear to transmitted light energy.



FINER SPECTRAL DEFINITION
Ten narrow band (40 millimicron) filters cover the entire visible spectrum. Two extra spaces are provided for special filters.

EASIER OPERATION
All adjustments made with a single control. Any competent laboratory worker can use the Photrometer, for no special skills are required.

The Instrument for *ANY* Colorimetric Test—the Rouy Photrometer is pre-calibrated for the 38 most commonly required clinical tests and can be calibrated for *any test scientifically suited to colorimetric technique.*

No Standards . . . No Calculation—Pre-calibration and an Individual Handbook eliminate any need for calculation or preparation of standards. Accurate reagents are quickly available through the Leitz Solution Supply Service.

Performance Proved—Photrometers and Leitz Colorimeters are now serving more than 8,300 satisfied users. Each instrument is made to exacting standards of precision and sturdiness by Leitz, first large-scale producer of photoelectric colorimeters.

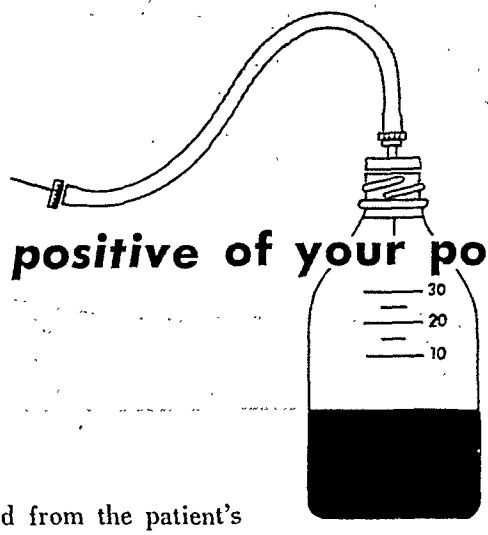
Pre-calibrated for 38 tests—$257.80
Pre-calibrated for 22 tests—$182.80

For information, write **E. LEITZ, Inc.,** 304 Hudson Street, New York 13, N. Y.

LEITZ SCIENTIFIC INSTRUMENTS • MICROSCOPES • BINOCULARS
LEICA CAMERAS AND ACCESSORIES

(In writing to advertisers, please mention the Journal—it helps.)

NOW



you can be *positive* of your positives

Direct delivery of blood from the patient's vein, through a closed, sterilized system, into Trypticase Soy Broth media in the new B-D VACUTAINER CULTURE BOTTLE . . .

- **Provides *positive* positives and valid negatives**
- **Prevents outside contamination**
- **Avoids incorrect selection and multiplicity of media**

Aerobic or anaerobic conditions may be obtained at time blood specimen is taken. Bottle reaches laboratory ready for immediate incubation . . . speeds laboratory handling . . . provides faster diagnosis. Microaerophiles grow luxuriantly.

Write for descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE and learn how easy it is to add this new element of assurance to your blood culture procedures.

with the

B-D

VACUTAINER®

CULTURE

BOTTLE

containing Trypticase Soy Broth with CO_2 added

Prepared and sterilized by Baltimore Biological Laboratories, Inc., Baltimore, Md.

BECTON, DICKINSON & CO., Dept. 2S-G
15 Ames Avenue,
Rutherford, N. J.

Please send me descriptive booklet on the new B-D VACUTAINER CULTURE BOTTLE that takes the guesswork out of blood culture procedures.

NAME_____

ADDRESS_____

CITY_____ ZONE____ STATE_____

BECTON, DICKINSON & CO., RUTHERFORD, N. J.

(In writing to advertisers, please mention the Journal—it helps.)

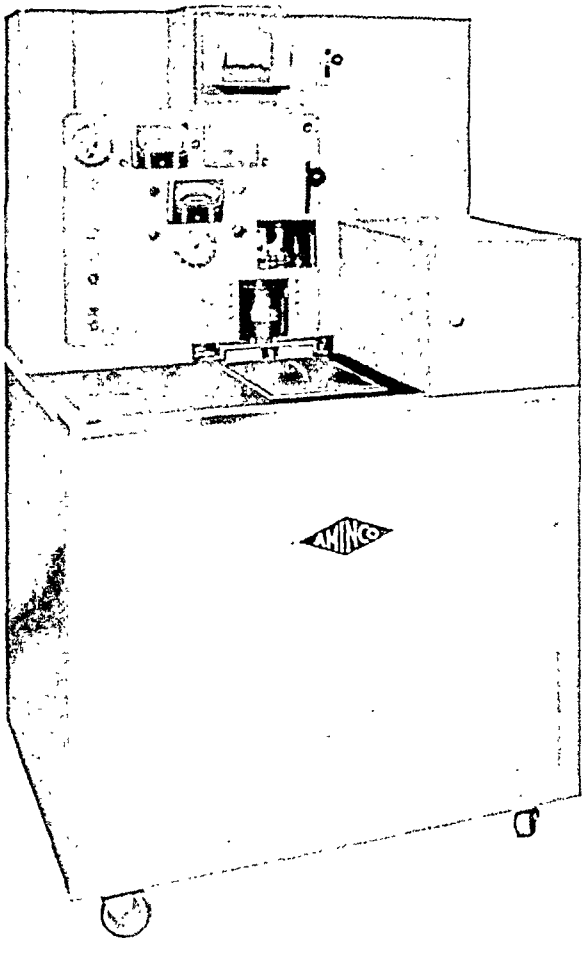
Far ahead of the field!

AMINCO STERN

ELECTROPHORESIS APPARATUS

it will pay you to investigate the MOST ADVANCED AND COMPLETE instrument for Electrophoretic analysis

Exclusive Features
NOT TO BE FOUND ELSEWHERE



- Most economical because of greater analytical capacity.
- Most useful for research and routine work.
- High sensitivity . . . can detect protein concentrations as low as 0.002%.
- Operates continuously regardless of atmospheric conditions, except for servicing.
- Most complete . . . includes rapid dialysis facilities, chamber for freezing and storing specimens, sealed mechanical refrigeration system, precision Schlieren optics, complete photographic facilities, and a rotatable triple diaphragm for selecting slit, bar and straightedge patterns.
- Compact . . . measures only 30 x 37 x 67 inches high. Moved about with ease.
- More convenient . . . operator works in front of the instrument. All parts at convenient heights and locations.
- Shipped ready for operation.
- Accommodates semi-micro, macro, macropreparative, adsorption, and conductivity cells.
- Engineered for long life. Components rated far beyond their normal operating requirements.

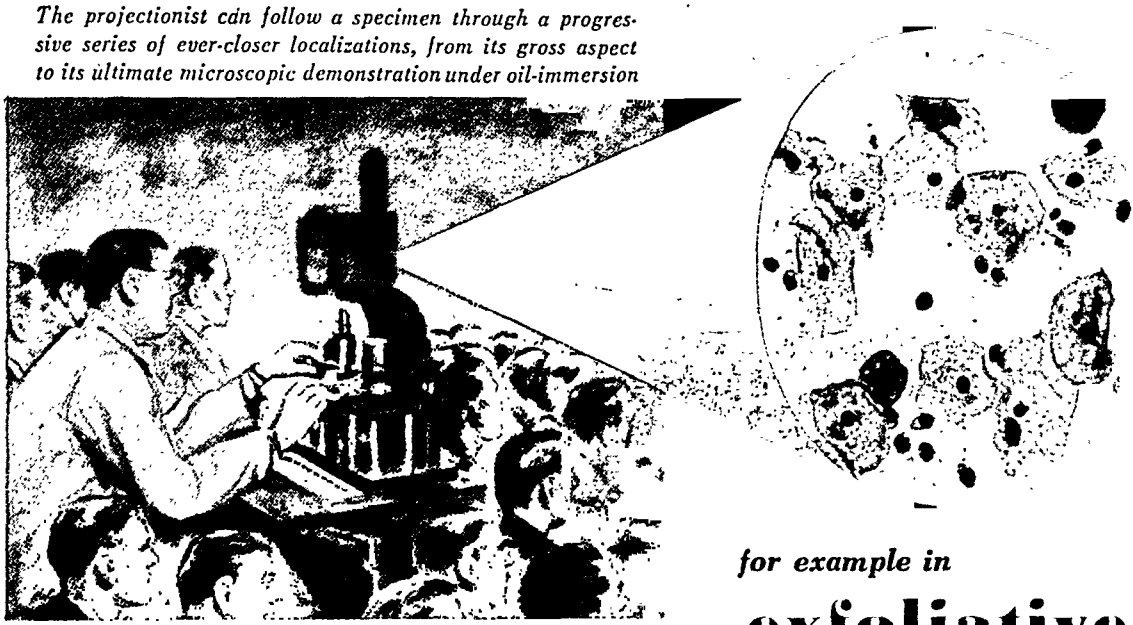
BULLETIN-2175P
TELLS THE ENTIRE STORY
Write for your copy

Since 1919 AMINCO

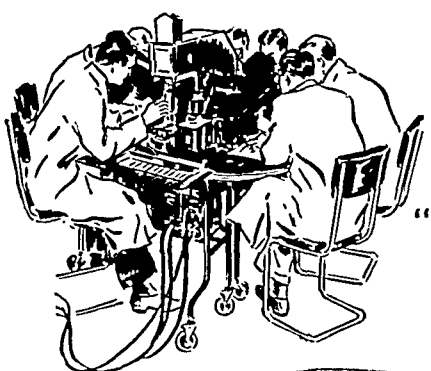
AMERICAN INSTRUMENT CO., Inc.
Scientific Instruments
SILVER SPRING, MARYLAND
IN METROPOLITAN WASHINGTON, D. C.

mass demonstration in the auditorium

The projectionist can follow a specimen through a progressive series of ever-closer localizations, from its gross aspect to its ultimate microscopic demonstration under oil-immersion



The auxiliary Scopicon dark-chamber "round-table" affords superb facility for small-group study. Here the projected image is cast upon a platen within a light-tight chamber, with separate light-excluding viewing hoods for each observer. Any number up to ten may share in conference, which can be conducted in a normally lighted room. A pointer at each port permits any observer to indicate areas of interest to all the others for discussion.



. . . and for small-group "round-tables" in a normally lighted room

for example in

exfoliative cytology

The 64,000 lumen intensity of the Scopicon high pressure mercury lamp permits auditorium demonstration of vaginal, uterine, bronchial and gastric smears and smears made from urine or other body exudates, even under oil-immersed microscope objectives. In projected images six feet or more across, every detail of the normal and abnormal cells can be seen due to the pinpoint character of the 1 mm. square (approx.) focal spot employed. The Scopicon light is steady, flickerless . . . its brilliant white color exhibiting biological stains to the greatest advantage. May we send you the brochure describing this versatile instrument?

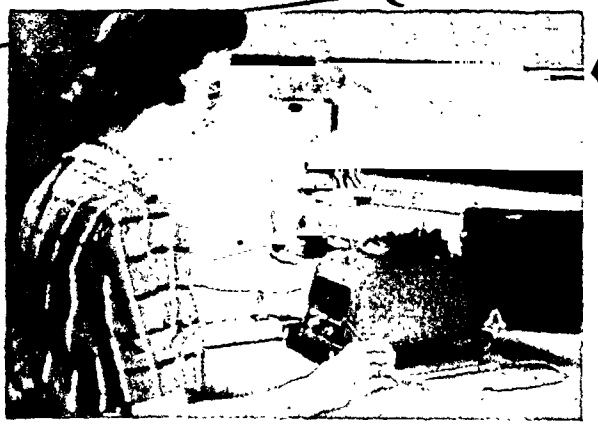
the Scopicon

microprojection photomicrography

SCOPICON, Inc.
215 East 149th St., New York 51, N. Y.

FISHER ELECTRO-HEMOMETER

Eliminates the Human Factor in Measuring HEMOGLOBIN



1. The Fisher Electro-Hemometer (Pat. Appl'd. for) can be quickly standardized by the operator by means of the water cell provided.

2. The water cell is replaced by the sample and its hemoglobin content is read directly from the scale.



Scale Indicates Directly Grams per 100 ml. and Per Cent Normal

The Fisher Electro-Hemometer, employed in the standard acid-hematin method, reads directly the hemoglobin in per cent, also in grams per 100 ml.

The results are obtained quickly and are more accurate than can be had by the older visual methods.

The instrument is portable, simple to operate, and the direct-reading feature eliminates the necessity of any calculations.

Electro-Hemometer, complete with instructions, ready for use.....$90.00

(Leather carrying case, $9.00 additional)

Manufacturers—Distributors

FISHER SCIENTIFIC CO.
717 Forbes St., Pittsburgh (19), Pa.
2109 Locust St., St. Louis (3), Mo.

EIMER AND AMEND
Greenwich and Morton Streets
New York (14), New York

Headquarters for Laboratory Supplies

we say they're better

and so will you when you've

proved it in your own laboratory

HYLAND BLOOD GROUPING SERUM

Anti-A and Anti-B

Prepared from carefully selected donors to assure a highly potent, rapid acting, and specific product. Accurate results with maximum speed.

Available in 2 cc. and 5 cc. sets.



HYLAND ANTI-Rh SERUMS

Anti-Rh_0 (Anti-D) Typing Serum

Anti-Rh_0 rh' rh" (Anti-CDE) Typing Serum

Outstanding serum for slide method—rigidly tested for titer, avidity, specificity and sterility. Prepared from selected, immunized donors.

Both types available in 2 cc. and 5 cc. sizes.



make this test at our expense!

Clip the coupon for your free samples today! You'll receive generous samples of Rh_0 Typing Serum and Blood Grouping Serum. Enough to convince you that their speed, accuracy and titer are unsurpassed.

Hyland Laboratories

4534 Sunset Boulevard, Los Angeles 27, California

Please send me generous samples of Rh_0 and Blood Grouping Serums.

Your Name_____

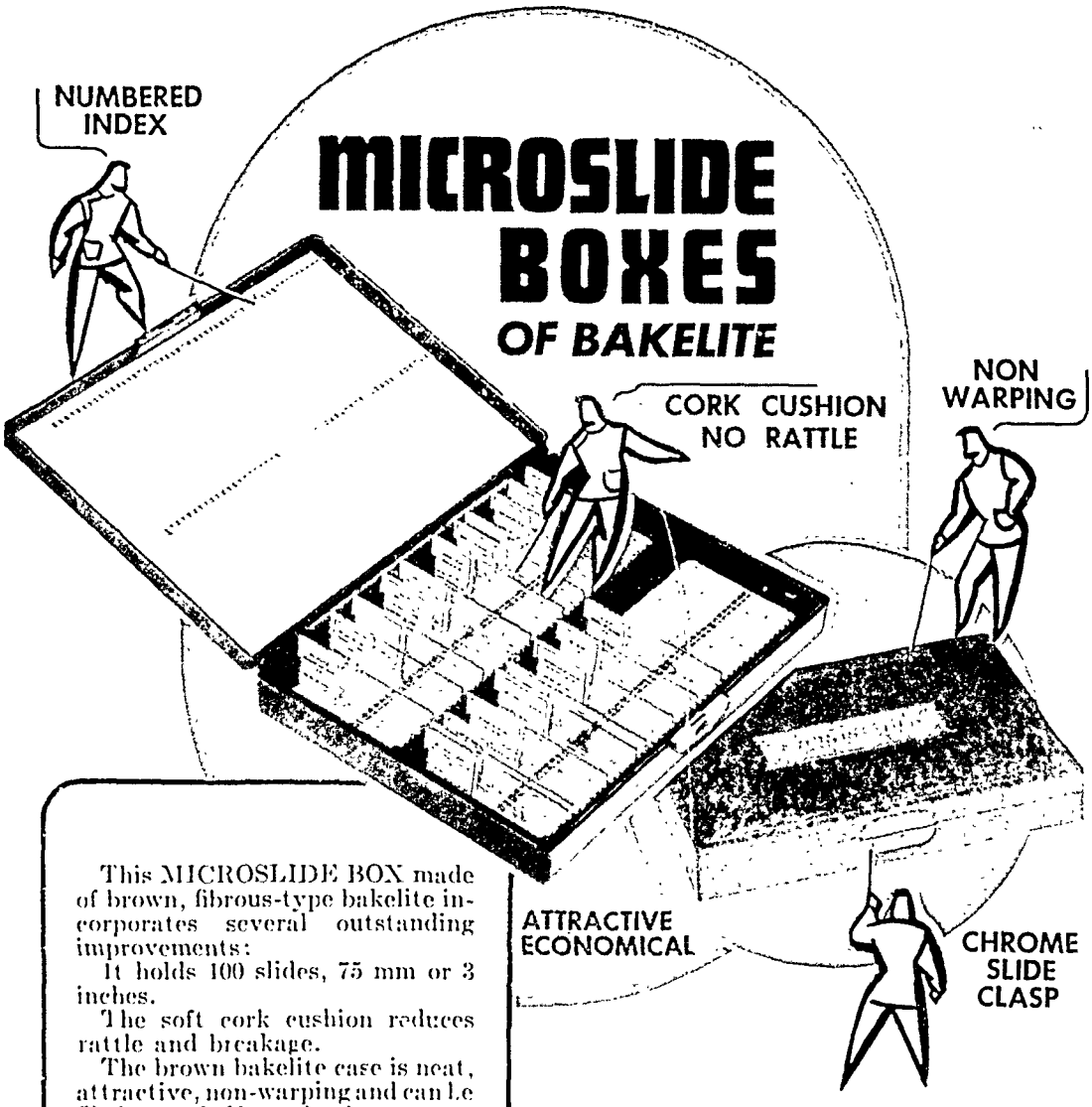
Hospital or Lab. Name_____

Address_____

City and State_____

From what dealer do you regularly buy your typing serums?_____

(In writing to advertisers, please mention the Journal—it helps.)



This MICROSLIDE BOX made of brown, fibrous-type bakelite incorporates several outstanding improvements:

It holds 100 slides, 75 mm or 3 inches.

The soft cork cushion reduces rattle and breakage.

The brown bakelite case is neat, attractive, non-warping and can be filed on a shelf as a book.

The numbered indexes in the lid and on the cork bottom of the slide compartment permit easy reference to slides.

A chrome slide clasp and large double hinges assure convenient opening and closing.

No. 66417 MICROSLIDE BOXES, dimensions 6½ x 8¾ x 1½ inches.

Each $2.05
Lot of 12 each 1.85
Lots of 36 each 1.74
Lots of 72 each 1.64
Shipment from stock.

CENCO

CENTRAL SCIENTIFIC COMPANY

Scientific Apparatus • Instruments • Chemicals

1700 IRVING PARK ROAD, CHICAGO 13

NEW YORK BOSTON SAN FRANCISCO NEWARK LOS ANGELES TORONTO MONTREAL

Announcing

ANTI-HUMAN PRECIPITIN SERUM

(rabbit)
for the "Coombs Developing Test"

a new and valuable diagnostic aid in

- Detection of infant sensitization with maternal Rh antibodies.[1,2,3,4]
- Detection of antibodies (blocking cryptagglutinoids) which coat but do not clump saline suspended cells.[1,3,4]
- Study and diagnosis of certain hemolytic anemias.[5]

packaging 2 cc vials with dropper cap assembly

a complete line of antiserums

more convenient Ortho Anti-Rh Sera are carefully processed from human bloods and possess excellent avidity, specificity, and titer. Ortho currently offers:

Anti-Rh_o (Anti-D) Slide Test Serum 4 cc pkg. (150 tests approx.)
Anti-Rh_o (Anti-D) Test Tube Test Serum 2 cc pkg. (75 tests approx.)
Anti-Rh_o' (Anti-CD) 87% Test Tube Test Serum 2 cc pkg. (75 tests approx.)
Anti-rh' (Anti-C) 70% Test Tube Test Serum 2 cc pkg. (75 tests approx.)
Anti-rh" (Anti-E) 30% Test Tube Test Serum 2 cc pkg. (75 tests approx.)

Further information and prices on request.

bibliography (1) Coombs, R. R. A., Mourant, A. E., and Race, R. R.: A New Test for the Detection of Weak and "Incomplete" Rh Agglutinins, Brit. J. Exp. Path. 26:255-266, 1945. (2) Coombs, R. R. A., Mourant, A. E., and Race, R. R.: In-Vivo Isosensitization of Red Cells in Babies with Hemolytic Disease, Lancet, 250:264-266, 1946. (3) Hill, J. M., and Haberman, Sol: Demonstration of Rh Antibodies in the Newborn and Further Evidence of the Pathogenesis of Erythroblastosis, J. Lab. and Clin. Med., 31:1053-1066, 1946. (4) Levine, P., and Wigod, M.: Technic of Rh Testing, "Diagnostic Procedures and Reagents," Am. Publ. Health Assoc. In Press. (5) Sturgeon, P.: A New Antibody in Serum of Patient's with Acquired Hemolytic Anemia. Science, 106:293-294, 1947.

Ortho Pharmaceutical Corporation • Raritan, New Jersey

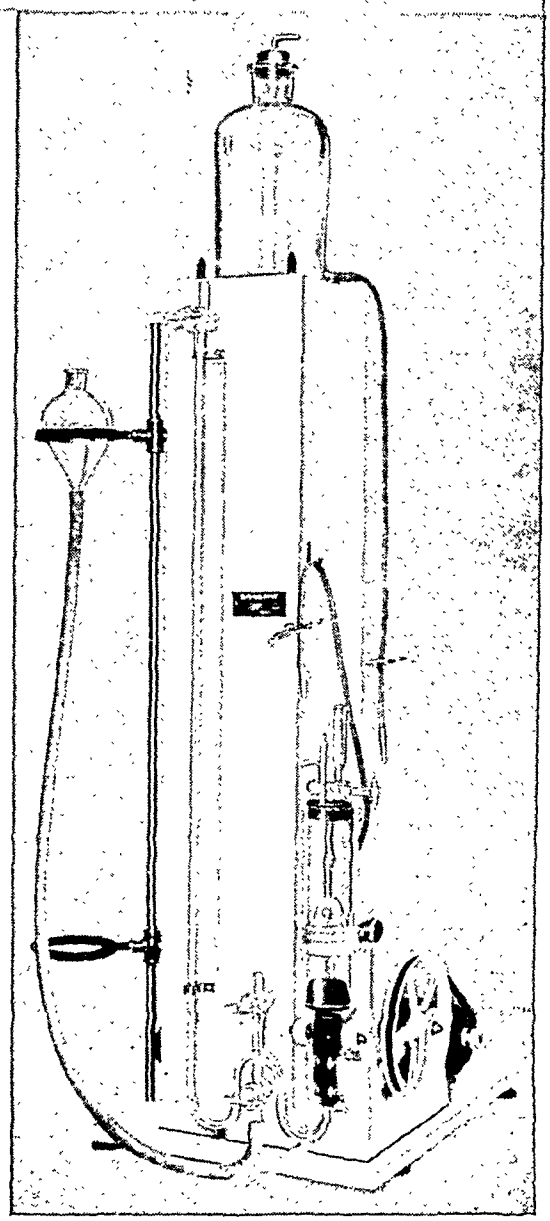
BLOOD GAS APPARATUS
(Manometric)
VAN SLYKE-NEILL

- ALL METAL CONSTRUCTION
- VIBRATION FREE
- SILENT OPERATION
- LONG SERVICE LIFE
- ALL CALIBRATION MARKS PLAINLY VISIBLE

The welded steel tower contains a non-glare fluorescent tube to illuminate the manometer. All calibration marks on the manometer and the extraction chamber are plainly visible. The base, carriage, pulley and carriage support are cast aluminum and the entire instrument is finished in corrosion resistant white enamel.

The manometer is mounted on the tower by means of spring steel clips which give it full support and still permit easy removal for cleaning. The end of the manometer leading to the extraction chamber is held firmly in place by a bracket and plate assembly which prevents the transmission of vibration from the shaking assembly. The plate is held in place by thumb screws—eliminating the need for tools when removing the manometer from the superstructure.

The shaking assembly is ball-bearing mounted, to give smooth silent performance and is controlled by a wide range stepless variable transformer.



S-7325 BLOOD GAS APPARATUS — Van Slyke-Neill, All Metal, Sargent. Complete with Glassware. For operation from 115 volt 50/60 cycle circuits **$250.00**

SARGENT

SCIENTIFIC LABORATORY EQUIPMENT AND CHEMICALS

E. H. SARGENT & COMPANY, 155-165 EAST SUPERIOR ST., CHICAGO 11, ILL.
MICHIGAN DIVISION 1959 EAST JEFFERSON DETROIT 7, MICHIGAN
SOUTHWESTERN DIVISION 5915 PEELER ST. DALLAS 9, TEXAS

C. I. No. 778

PHLOXINE B

Certified for use in: Histology

..... GMS

Pharmaceutical Laboratories

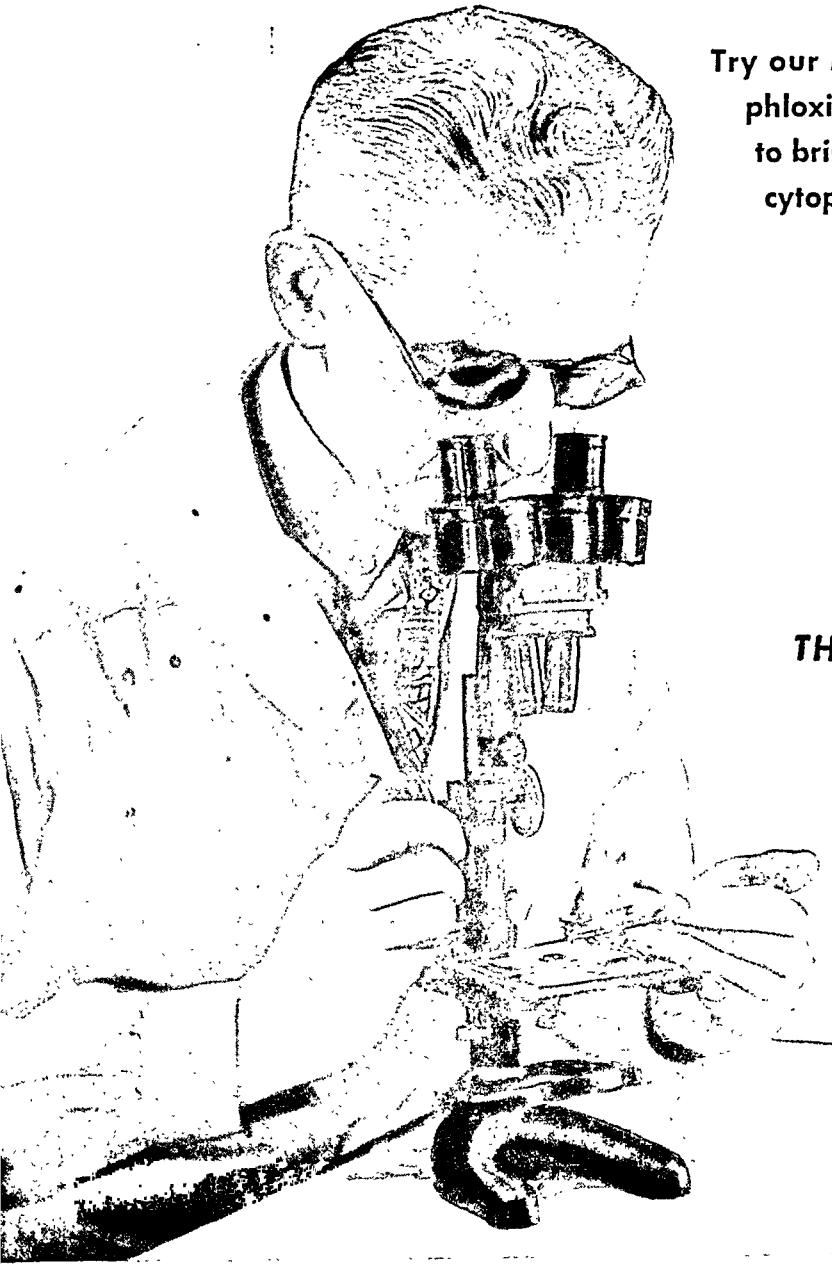
NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A.

Found satisfactory by BIOLOGICAL STAIN COMMISSION for above mentioned purposes. *This does not imply approval for any medicinal use.* Will users please report any unsatisfactory results to *H. J. Conn, Geneva, N. Y.*

Total Dye Content%

Certification No.
NPh



Try our *PHLOXINE B* in Mallory's phloxine methylene blue technic to bring out the pink color of the cytoplasm.

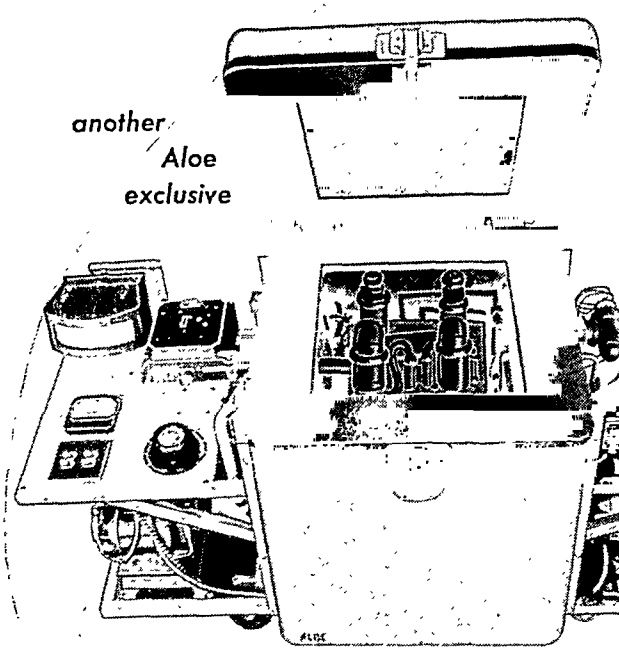
National

THE COMPLETE LINE OF
COMMISSION CERTIFIED BIOLOGICAL STAINS

Pharmaceutical Laboratories

NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A.



another Aloe exclusive

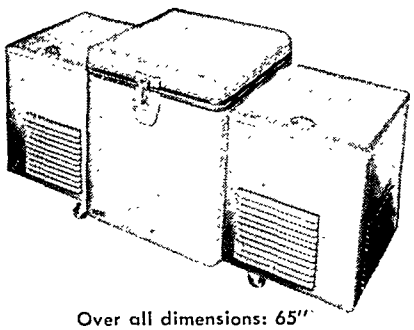
freezing-dehydration apparatus

for cell chemistry and pathology

New . . . Versatile . . . Compact. Built for exact studies. Aloe's flexible, stainless steel unit is based on the principles of the Altmann-Gersh freezing-drying apparatus and used by other recognized authorities. Important engineering improvements include direct reading scales and manual controls of both temperature and vacuum.

Under accurate controls, the new Aloe Freezing-Dehydration Apparatus, permits tissue to be dehydrated and fixed with minimal losses and alterations. Tissue, after freezing in isopentane, is placed in one of the vacuum chambers in refrigerating compartment. Temperature is accurately adjusted from —15°C to —50°C and vacuum as low as 0.001 mm mercury. If desired, the specimen is infiltrated with paraffin or cellodin. Sections of embedded materials may be used for morphological and cytochemical studies by numerous methods.

71580 FREEZING—DEHYDRATION APPARATUS, as described, with four vacuum-dehydration chambers, two desiccant tubes, and one Dewar flask. For 115 volts, 60 cycles, A. C. each.....$2,250.00



Over all dimensions: 65" wide x 28½" deep x 34½" high with covers closed and 56½" high with covers open.

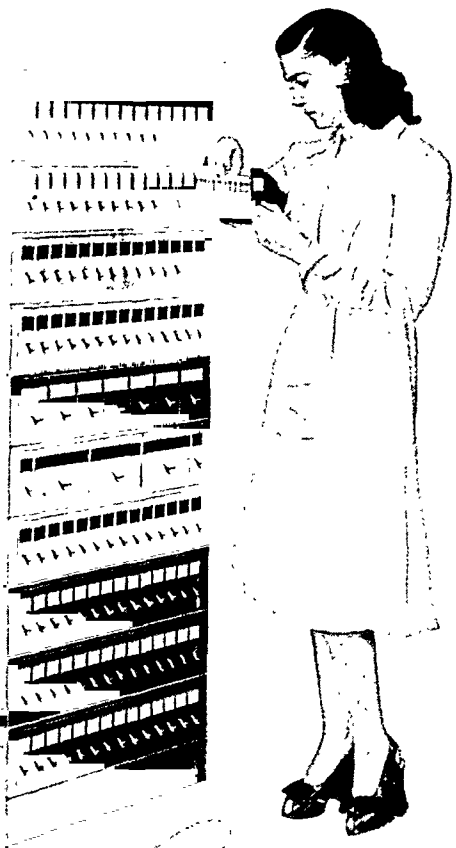
Write for illustrated booklet and bibliography — Bulletin No. T112

A. S. ALOE COMPANY *Serving science since 1860.*

1831 Olive Street • St. Louis 3, Missouri

aloe

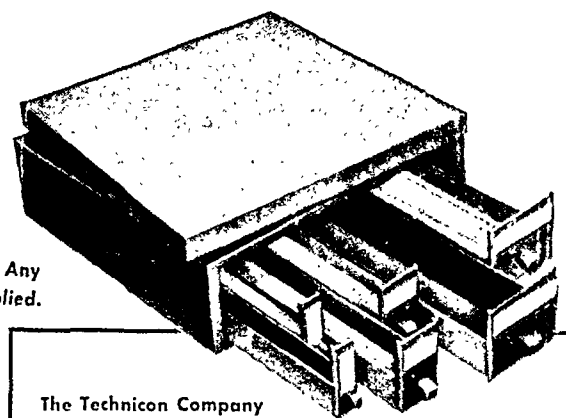
a steel microslide filing cabinet offering



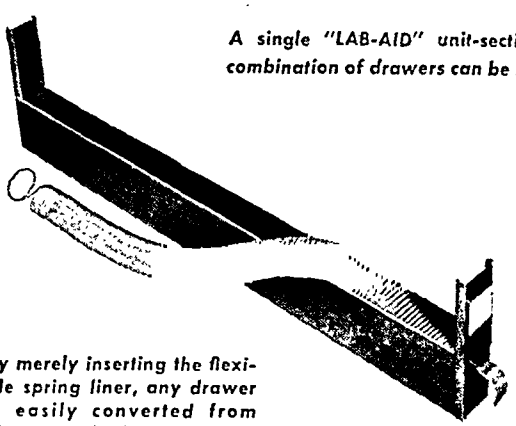
45% greater capacity at amazingly low cost!

This new "LAB-AID" cabinet is the *ultimate* solution to the filing problems encountered in the laboratory. It is of welded-steel, fire-resistant construction throughout. Files 3"x1" or 3"x2" microslides, 2"x2" transparencies, large lantern slides, index cards, even paraffin blocks . . . in single-row drawers, all fitting *interchangeably* into a smooth-tracking master drawer-rail system. Unit-sections, assembled in any combination, can be used singly, or stacked to any convenient height. With all these advantages, the *cost-per-slide* capacity in the "LAB-AID" cabinet is the *lowest* ever. Investigate this remarkable equipment . . . the coupon will bring details.

the new steel laboratory filing cabinet



A single "LAB-AID" unit-section. Any combination of drawers can be supplied.



By merely inserting the flexible spring liner, any drawer is easily converted from close packed storage to separate slide filing.

THE **technicon** COMPANY

The Technicon Company
215 East 149 Street
New York 51, N. Y.

Please send me details concerning your "LAB-AID" microslide filing cabinet.

Name_____

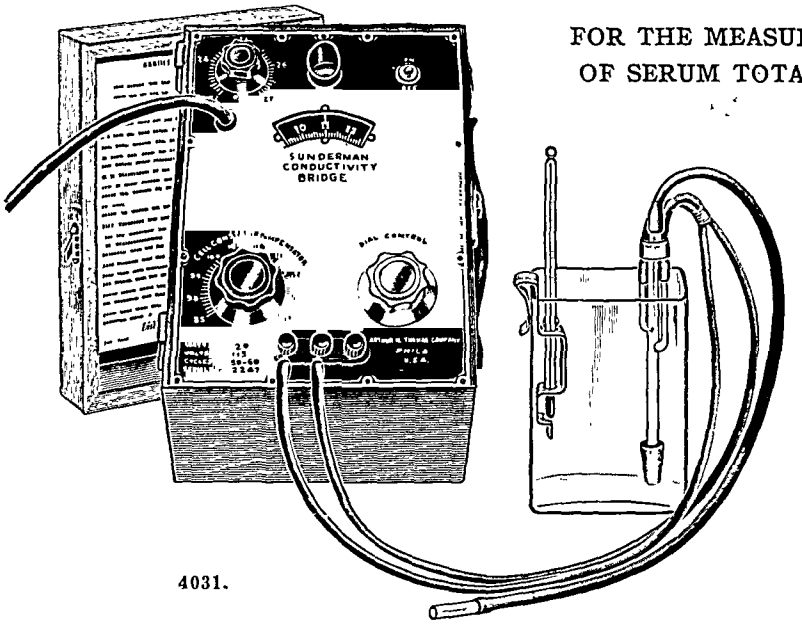
Street_____

City_____

SUNDERMAN DIRECT READING

CONDUCTIVITY OUTFIT

FOR THE MEASUREMENT
OF SERUM TOTAL BASE



4031.

CONDUCTIVITY OUTFIT. Sunderman Direct Reading Type, A.H.T. Co. Specification. For rapid routine determinations of the electrometric conductivity of small quantities of biological fluids, and particularly for the measurement of serum total base and the estimation of serum sodium. Tests can be made with only 1.5 ml of sample.

The Bridge is an improved, direct reading model of that originally described by F. William Sunderman, *American Journal of Clinical Pathology, Vol. 15, No. 6 (June, 1945), p. 219.* It is of the Wheatstone type, with direct reading dial graduated 5 to 21 in 0.1 divisions, indicating specific conductance expressed as mhos (reciprocal ohms) x 10^{-3} at 25°C. It is furnished with temperature cell constant compensators and "magic eye" null point indicator, and is entirely self-contained, requiring only the connection of the conductivity cell.

The Conductivity Cell, pipette form, is convenient for the collection of samples from small-mouthed vessels and for subsequent recovery of the sample for further tests. Each cell is individually calibrated and permanently marked with cell constant.

4031. **Conductivity Outfit, Sunderman Direct Reading Type,** consisting of Conductivity Bridge and Conductivity Cell, as above described, Water Bath 2½ x 4¼ x 6 inches, Thermometer Holder of Stainless steel, and Thermometer 0 to 40°C in 0.2° divisions. Complete with directions for use and author's nomogram for calculating serum total base, 5 ft. cord and plug. For 115 volts, 50 or 60 cycles, a.c. 149.00

4031-B. **Conductivity Bridge, Sunderman Direct Reading Type,** only, as supplied with above outfit, with directions for use and author's nomogram. For 115 volts, 50 or 60 cycles, a.c. 105.00

4031-E. **Conductivity Cell, Pipette Form, Sunderman Improved Type,** as supplied with above outfit, with platinized electrodes, 30-inch leads, glass bead valve, 20 inches of rubber tubing, mouthpiece and rubber closure for tip. With directions for use and author's nomogram 34.45

More detailed information sent upon request.

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

CABLE ADDRESS, "BALANCE", PHILADELPHIA

MEDICOLEGAL EXAMINATION OF BODIES RECOVERED FROM BURNED BUILDINGS*

FRANK R. DUTRA, M.D.

From the Office of the Coroner of Hamilton County, Ohio, and the Kettering Laboratory of Applied Physiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio

Conflagrations of medicolegal interest include those that may conceivably have been set to commit or conceal homicide or suicide, and those in which undue hazard to the public welfare may have contributed to death.

Most deaths caused by, or occurring in association with, fire are accidental, but some deaths which appear to be accidental have actually been planned. It is not uncommon for a murderer to attempt to conceal his crime by burning the scene and the body together. Persons sometimes set their premises afire before committing suicide in the hope that death will be accepted as accidental. Occasionally, persons commit suicide by igniting inflammable substances with which they have covered themselves.

When homicide is perpetrated by incineration, the victim is almost invariably first rendered unconscious by alcohol, drugs, or injuries. However, in a recent exception to this rule, a robber, apparently intending to prevent chase, threw a bucketful of gasoline and then a lighted match, into a small store, fatally burning the proprietor and his son.

In the hope of perpetrating a fraud, an attempt is occasionally made to have a death from natural causes appear as having accidentally resulted from a conflagration. The true cause of death can usually be determined by complete autopsy even though the remains have been severely burned. It is not uncommon for bodies recovered from burned buildings to be charred (Fig. 1), and for the extremities and head to have been partially burned away. Even then, it is usually possible to examine the body cavities and viscera in the ordinary manner.

In addition, blood may be collected for determination of carbon monoxide content in almost every case. When the body has been subjected to intense heat, the blood is sometimes completely coagulated and cooked. In such cases, masses of the coagulum should be retained in a tightly stoppered bottle. The presence of carbon monoxide in such blood can sometimes be determined, in the usual way, after first pulverizing the mass under oil.

DID DEATH PRECEDE, OR RESULT FROM, CONFLAGRATION?

From the autopsy, the examiner should attempt to determine whether or not the decedent had been alive at the start of the fire. Surface burns should be examined for evidences of a vital reaction, such as a bright line of localized cutaneous hyperemia at the edge of a burn. True blisters form only during life and contain fluid rich in protein. They are surrounded by thin, bright red zones of

*Received for publication, March 11, 1949.

hyperemia and, when the epidermis covering the blister is removed, the underlying dermis is also found to be bright red.

In some cases where death has occurred after the onset of the fire, there will be deposits of soot in the larynx and trachea (Fig. 3). Sometimes only a few small granules of soot are found, but occasionally the entire tracheobronchial tree is lined with this material, depending on the amount of soot present in the smoke of the conflagration.

Some victims of conflagrations develop numerous small visceral hemorrhages, which are usually most marked beneath the endocardium of the septal surface of the left ventricle of the heart (Fig. 4). Experiments have demonstrated that such hemorrhages occur within a few minutes after exposure to high temperatures, even when the possibility of inhalation of heated air or of noxious gases has been excluded,[4] thus supporting the belief that hyperthermia is the cause of the hemorrhages.

Perhaps the best evidence that a person was alive during a conflagration is the presence of excessive quantities of carbon monoxide in his blood. This substance is a by-product of nearly every fire, and chemical examination of blood for its presence is essential to the investigation of any death where conflagration may have been a factor.

It is usually also wise to examine the blood for ethyl alcohol, since this substance plays a part in a considerable proportion of deaths in conflagrations. The amount of alcohol in the blood may explain why the person was unable to escape from the flames. Furthermore, many fires result from the careless acts of inebriated persons.

The attitude of partially incinerated bodies is frequently one of semi-flexion of trunk and extremities (Fig. 1) and has been called the "pugilistic attitude". This posture has no significance in determining whether or not the person died as a result of the fire. It results from coagulation of the proteins of the skeletal muscle; the contraction is so marked that the muscles will be torn if the limbs are forcibly extended. If rigor mortis has not begun before such heat contraction takes place, rigor mortis will not set in later. On the other hand, if rigor mortis has already appeared and disappeared, exposure of the body to heat results in the establishment of heat rigor, unless putrefaction had already begun.[2] With the onset of the putrefaction in muscles which are the seat of heat rigor, there is gradual disappearance of the rigor.

ARTEFACTS OF INCINERATED BODIES

Certain artefacts resulting from heat may be confused with pre-mortem injuries. The most common artefacts of this type are splits of the skin which may resemble knife slashes, stab wounds, or lacerations (Fig. 2). The absence of hemorrhage in the tissues adjacent to these splits is sufficient to differentiate them from injuries sustained before death. Occasionally, intact blood vessels extend across such a split.

Pseudo-blisters are sometimes produced by heat after death. These result from a short exposure of the skin to a high temperature, so that steam forms in





FIG. 1. Body removed from burned building. Positive identification was established through the presence of gold open-faced crowns of the two upper central incisors which were readily recognized by relatives. The man had died during the fire, since there was soot adherent to the lining of the trachea and bronchi, proving that he had breathed smoke. Furthermore, examination of the blood revealed 52 per cent saturation with carbon monoxide. The extremities of partially burned bodies are often fixed in semiflexion.

FIG. 2. Numerous splits of the skin of back, resulting from intense heat. These sometimes simulate pre-mortem incised wounds or lacerations.



3



4

the dermis and elevates the epidermis. Such pseudo-blisters contain only gas, as contrasted with true blisters which contain liquid. There is no hyperemia around the periphery of a pseudo-blister and the underlying dermis is ordinarily yellow or gray rather than red.*

Postmortem fractures of the skull may occasionally result from the expansion of steam within the cranial cavity. These are usually found in the vault of the skull, and may be differentiated from pre-mortem injuries by the separation of the edges of the fracture by the escape of the steam. Furthermore, traumatic fractures of comparable size are almost invariably accompanied by adjacent epidural hemorrhage and may be associated with lacerations and contusions of the brain substance.

A portion of the scalp is sometimes burned away exposing the outer table of the skull to direct heat and desiccation. The bone becomes brittle and shrinks as the drying progresses, leading to the development of cracks in the outer table of the skull which simulate fracture lines (Fig. 5).

It is not uncommon to find coagulated pinkish brown blood between the skull and dura mater after a body has been partially incinerated. Such postmortem extravasations form a single large clot which extends over the vertex and both parietal regions (Fig. 6). These clots differ from traumatic epidural hemorrhages, in that the latter are usually confined to one temporoparietal region and, even when extensive, do not cross the vertex. Also, traumatic epidural hemorrhages are nearly always associated with fractures of the skull.

The partially incinerated bodies of persons known to have been well immediately before a fatal fire are often found to have red, boggy, hyperemic lungs. The lungs contain blood within their alveoli, and occasionally the bronchi and trachea are also filled with blood. The source of this blood is unknown, but it is believed that as heat contracts the muscles throughout the body, the pressure on the systemic veins may express the blood through the chambers of the right side of the heart and into the pulmonary artery. As a result, there is engorgement and finally rupture of the pulmonary capillaries with hemorrhages into the lung tissue.

Occasionally, persons will be subjected to great heat without actually coming into contact with flames. The author has observed this phenomenon in three instances where the victims had been exposed to escaping steam. In such cases death may occur, and the skin and superficial tissues may be completely cooked without great alteration of the appearance of the body. A superficial examination might lead to the conclusions that the body had not been subjected

*Another form of pseudo-blister seen in medicolegal work is not related to conflagrations. This is the bleb which appears early in putrefaction, and which contains fluid but has no peripheral zone of hyperemia.

FIG. 3. Trachea and major bronchi with soot adherent to mucosa, indicating that smoke had been inhaled immediately prior to death. Death in conflagration.

FIG. 4. Subendocardial hemorrhages, septal surface of left ventricle, due to hyperthermia. Heart of man who died 15 minutes after he had been removed from a burning building. There were extensive second and third degree burns of the body.

to heat, and that the discoloration of the skin was the result of putrefactive changes. However, dissection reveals that the collagen of the dermis is swollen and friable because it had been cooked. Conversely, in early putrefaction, the dermis is not friable, since its collagen resists putrefaction.

LETHAL FACTORS IN CONFLAGRATIONS

There are several potentially injurious factors in most fires and any one, or any combination, of them may cause death. These factors include heat, falling structural materials, and noxious gases, smokes and fumes. The possibility that depletion of oxygen might cause death is also considered below.





FIG. 5. Postmortem cracks of the outer table of the skull caused by heat and desiccation, simulating pre-mortem fractures.

FIG. 6. Postmortem epidural blood clot, the result of intense heating of the head.

When death has been caused by heat, there are usually extensive burns of the body, and the clothing is usually partly or completely consumed. The skin may be burned superficially or charred completely with considerable destruction of the underlying tissues.

Death by hyperthermia may occur without significant damage to the skin. Experiments[4] have shown that exposure of young pigs (7–12 Kg.) to air temperatures of 90 C. for 45 minutes will produce death, yet the epidermis will not be destroyed. Deaths of this type should probably be considered as acute heat stroke. On the other hand, young pigs cannot be exposed for more than 30 seconds to air at 600 C. without generalized destruction of the skin and death from hyperthermia. The exact conditions which would produce similar results in

man are not known, but it is believed that these samples represent conditions existing in some conflagrations in which people are killed or injured.

The appearance of pulmonary edema in persons injured in some conflagrations has been thought by some[8] to result from thermal injuries of the lungs. The work of Moritz, Henriques, and McLean[5] has proved that the capacity of dry gases to carry heat into the bronchi and lungs is not great enough to account for injury of these structures, so that one or more other mechanisms must be responsible for such damage. It is now recognized that noxious products of the thermal decomposition of various materials in conflagrations cause the pulmonary lesions.

Many victims of conflagrations inhale gases, fumes and smokes, some of which are highly toxic. These substances are produced in greatest quantity, and with greatest rapidity, when the temperature is relatively high and the oxygen supply is limited as in burning buildings. On the other hand, it is uncommon for anyone to be overcome by gases from fires which are burning in the open. Experiments[2] have been conducted to demonstrate these points, using artificial leatherette that was recovered from the unburned walls after the conflagration at the Boston Cocoanut Grove Club in 1942. When this leatherette was burned in a current of fresh air, the smoke produced only slight irritation of the respiratory tracts of rabbits; but when, in the absence of a supply of fresh air, the same material was decomposed by applying the flame to the flask containing dry leatherette, irritating gases were evolved which killed the animals within as short a time as 100 minutes from onset of exposure.

The most important and usually the most dangerous of the toxic gases in conflagrations is carbon monoxide, because of its ubiquitous occurrence and insidious action. It is impossible to determine with accuracy the number of deaths in fires which are largely or entirely due to carbon monoxide, but there can be little doubt that a considerable proportion of them are directly attributable to this gas. Only rarely does the blood of a victim fail to contain a large quantity of carbon monoxide, regardless of whether the body has been only slightly burned or almost completely consumed.

The amount of carbon monoxide in the blood which will produce death probably varies somewhat with the health, age, and with the activity of the person during and immediately after exposure to the gas. Search of the literature has revealed no record of a study of these factors, but it is believed that young children, old persons, and those suffering from lung or heart disease (diminished pulmonary or cardiac reserve) would probably die with less carbon monoxide in the blood than would be required to kill healthy young adults.

It is considered unlikely that the depletion of atmospheric oxygen causes deaths in conflagrations, since after enclosed fires are allowed to die out the oxygen remaining in the atmosphere is sufficient to support life, whereas the carbon monoxide level is incompatible with life.[4]

Carbon dioxide cannot be considered dangerous, since in enclosed fires lethal levels of carbon monoxide form more rapidly.

Other highly toxic gases are also evolved in some conflagrations, including acid oxides of nitrogen, sulfur oxides, hydrogen sulfide, hydrogen cyanide and acrolein.

The possibilities depend on the materials being burned. Dangerous quantities of nitrogen dioxide and nitrogen tetroxide are liberated by the burning of nitrogen-containing materials such as nitrocellulose film,[7] and artificial leatherette.[2] The burning of wool or silk liberates ammonia and hydrogen cyanide.[6] The burning of wool and other sulfur-containing substances also yields oxides of sulfur as well as hydrogen sulfide. Acrolein is liberated in toxic quantities by the thermal decomposition of neutral fats and other materials containing glycerine. This highly irritating compound has been found to be a product of the thermal decomposition of a sizer oil which was extracted from artificial leatherette removed from the Cocoanut Grove Club, and it may have contributed to the pulmonary injuries of some of the victims of that disaster.[2]

IDENTIFICATION OF BURNED BODIES

Even though a body has been severely burned, it is usually possible to establish identification.

Clothing or jewelry on the body may aid, but it should not be forgotten that anyone wishing to confuse identification might substitute these articles before a fire is started. Important information relating to identity can be obtained from the teeth, which can be compared with records obtained from the dentists of persons known to be missing. The organs and skeleton should be carefully examined for evidence pertaining to the race, sex, and age of the body. Individual peculiarities, such as evidences of pre-existing disease or deformities and absence of certain organs as a result of surgery, should be recorded. X-ray examination of the entire skeleton may be of considerable value.[1]

PROTECTION OF THE PUBLIC WELFARE

The medical investigator has the opportunity of serving the public interest when there has been a conflagration with loss of life. Even though criminal negligence is not suspected, autopsy should be performed to establish the exact cause of the death of the victim, and in multiple deaths, of a significant number of victims. In this way it may be determined if toxic gases or other unsuspected factors played a part in the deaths, and the information obtained may be of value in treating injured survivors, or in subsequent investigations by fire and police officials.

SUMMARY

1. The examination of a body recovered from a burned building should reveal if the individual was alive or was dead at the onset of the conflagration. If death has occurred in a fire, the blood will almost invariably show a high level of carbon monoxide. In addition, particles of soot are usually present on the mucosae of the larynx, trachea, and bronchi. The presence of true blisters, or of a peripheral zone of hyperemia, associated with burns of the skin indicate life at the time the burns were sustained.

2. If death occurred in the fire, an analysis of the blood for ethyl alcohol may reveal that escape was rendered more difficult by inebriation. Examination of the viscera and extremities will reveal whether injury or disease prevented escape.

3. Certain postmortem artefacts of burned bodies, produced by heat, may be confused with pre-mortem injuries. These artefacts include splits of the skin which simulate incised wounds, pseudo-blisters caused by formation of steam beneath the epidermis, cracks of the outer table of the skull from drying and shrinkage, fractures of the entire thickness of the skull from the generation of steam in the cranial cavity, and epidural hematomas over the vertex of the brain.

4. Most deaths in conflagrations are due to heat, noxious gases (especially carbon monoxide), or the combined effects of heat and gases. Death from heat is indicated by extensive burns of the body, together with hemorrhages beneath the endocardium of the septal surface of the left ventricle of the heart.

5. Identification of partially burned bodies may be made from clothing or jewelry, or dental peculiarities, or from individual physical characteristics (congenital abnormalities, or residua of disease or surgery).

REFERENCES

1. Dutra, F. R.: Identification of person and determination of cause of death from skeletal remains. Arch. Path., **38**: 339–349, 1944.
2. Dutra, F. R.: Unpublished observations.
3. Forbes, W. H., Sargent, F., and Roughton, F. J. W.: The rate of carbon monoxide uptake by normal men. Am. J. Physiol., **143**: 594–608, 1945.
4. Moritz, A. R., Henriques, F. C., Jr., Dutra, F. R., and Weisiger, J. R.: Studies of thermal injury; exploration of the casualty-producing attributes of conflagrations; local and systemic effects of general cutaneous exposure to excessive circumambient (air) and circumradiant heat of varying duration and intensity. Arch. Path., **43**: 466–488, 1947.
5. Moritz, A. R., Henriques, F. C., Jr., and McLean, R.: The effect of inhaled heat on the air passages and lungs. Am. J. Path., **21**: 311–331, 1945.
6. Olsen, J. C., Ferguson, G. E., and Sheflan, L.: Gases from thermal decomposition of common combustible materials. Indust. and Engin. Chem., **25**: 599–603, 1933.
7. von Oettingen, W. F.: The toxicity and potential dangers of nitrous fumes. Public Health Bull., 272, 1941.
8. Wilson, W. C., Macgregor, A. R., and Stewart, C. P.: The clinical course and pathology of burns and scalds under modern methods of treatment. Brit. J. Surg., **25**: 826–865, 1937–38.

STUDIES ON INCLUSION BODIES

I. Acid-Fastness of Nuclear Inclusion Bodies That Are Induced by Ingestion of Lead and Bismuth*

M. WACHSTEIN, M.D.

From the Laboratories of St. Catherine's Hospital, Brooklyn, New York, and the Division of Pathology, Mt. Sinai Hospital, New York, New York

Inclusion bodies may occur in the cytoplasm of the cell, the nuclei or both. For some inclusion bodies, as in vaccinia, a virus origin has been established beyond doubt; for others, particularly those that follow the application of certain chemical substances, such a causal relationship is unlikely. For the inclusion bodies which occur after the injection of aluminum and ferric compounds, Olitsky and Harford[22] excluded the possibility of a virus infection by transmission experiments. Although much work has been done to trace the origin of the various inclusion bodies to certain normal cellular constituents by various stains, including histochemical tests, as well as by centrifugation procedures (Lucas[15]), the histogenesis and chemical composition of many of the inclusion bodies is still doubtful.

The present work was first undertaken in order to study a number of histochemical reactions in nuclear inclusion bodies that occurred after the ingestion of lead in rats. When marked acid-fastness of these inclusion bodies was noticed, various other inclusion bodies were examined for this property.

MATERIAL AND METHODS

Lead. Eleven young white albino rats of the Wistar strain, each weighing approximately 100 Gm., were given a diet composed of 59 per cent dextrose, 19 per cent casein, 10 per cent lard, 5 per cent yeast, 4 per cent salt mixture, 2 per cent cod liver oil and 1 per cent choline chloride. The diet contained 4 Gm. lead acetate per kilogram. Two of the animals died after 56 and 92 days, while the remaining 9 were killed after 142 days. Two control groups of 10 animals each were given a similar diet which in one instance contained 2.5 Gm. nickel nitrate per kilogram, and in the other instance contained 1 Gm. cobalt chloride per kilogram. The animals on cobalt chloride died or were killed after 90 to 95 days.

Bismuth. A block of formalin-fixed human kidney tissue (autopsy № 12933, Mt. Sinai Hospital) was available for study. This case had been previously used for the histochemical identification of bismuth in the colon as well as in the renal inclusion bodies by Wachstein and Zak.[31] The patient was a 30 year old white woman who had a history of rheumatic fever and chorea in childhood, and hypertension two years prior to her death. Her blood Wassermann reaction was 4 plus and she was treated with weekly bismuth injections for one year. She died two days after a sudden onset of severe dyspnea and cyanosis. Necropsy revealed rheumatic heart disease, malignant nephosclerosis and bismuth melanosis.

* Received for publication, January 25, 1949.

Additional material examined. The following tissues containing inclusion bodies were also available for study:

1. Lungs of a woman with chronic pneumonitis (autopsy #12617, Mt. Sinai Hospital)
2. Salivary glands of a child who died from acute enteritis (autopsy #13389, Mt. Sinai Hospital)
3. Liver and kidneys of a newborn child (courtesy Dr. P. Gruenwald, Department of Pathology, Long Island College of Medicine, Brookyln, New York)
4. Beta cells of the chicken pancreas (courtesy Dr. A. M. Lucas, U. S. Regional Poultry Research Laboratory, East Lansing, Michigan)
5. Intestinal epithelium of mice with epidemic diarrheal disease (courtesy Dr. A. Pappenheimer, Harvard Medical School, Boston)
6. Negri bodies in dog brain (courtesy Dr. J. Schleistein, Division of Laboratories and Research, Albany, New York)
7. Bladder of a mink with distemper (courtesy Dr. J. R. Gorham, U. S. Fur Animal Disease, Research Laboratory, Pullman, Washington)
8. Testicle of a rabbit infected with Virus III (courtesy Dr. J. M. Pearce, Cornell University Medical College, New York)

Staining procedures. Sections from formalin-fixed material gave the best results. Good results were also obtained on material fixed in Zenker's solution, after the usual treatment with iodine and sodium thiosulfate. The sections were put in Ziehl-Neelsen's carbol fuchsin at 56 C., for three hours. They were then washed, decolorized for three to five minutes in acid-alcohol (3 ml. concentrated hydrochloric acid and 97 ml. of 70 per cent alcohol) and counterstained with Harris' hematoxylin. Sections were also stained with hematoxylin and eosin and in addition, special stains were occasionally used.

RESULTS

Lead. In all animals that were given lead, numerous nuclear inclusion bodies were found in the kidneys; none were seen in the liver. The inclusion bodies closely resembled those that were first described by Blackman in the kidneys and livers of children who died of lead poisoning and also in rats that were given lead in their food.[2] Similar observations in experimental animals were later made by Finner and Calvery,[9] Diaz-Rivera and Horn,[5] and Dalldorf and Williams.[4] In sections stained with hematoxylin and eosin, these inclusion bodies were usually sharply outlined and homogeneous. Nearly all of them were eosinophilic, although an occasional one stained purplish. They were round or irregular in shape and varied in size from tiny droplets to bodies larger than the normal nuclei. One or several inclusion bodies were found in one nucleus. The large inclusion bodies were usually centrally located while smaller ones were often found in the periphery of the nucleus. The nuclei in which inclusion bodies were present were frequently enlarged. The chromatin was coarse and granular and was found either located on the outer surface of the inclusion body or had marginated to the nuclear membrane. Occasionally chromatin was present in both locations in the same nucleus.

Inclusion bodies were found only in the cortex and in the subcortical zone and not in the inner portion of the medulla. They were about equally numerous in the outer zone of the cortex and the subcortical zone. They were found in tubules that could be easily identified as proximal convolutions but occurred also

in tubules forming the medullary rays. The latter predominantly contain branching collecting tubules and straight thick portions of ascending limbs of Henle's loops. A more exact localization will have to await the isolation of single renal tubules as advocated by Oliver.[23] (See also Wachstein.[30])

In addition to the occurrence of inclusion bodies, definite changes were found in the cortical tubules. Some of the cells showed large swollen vesicular nuclei as well as degenerative changes in the cytoplasm. These degenerative changes had progressed in some of the tubules to frank necrosis with sloughing of tubular epithelium, and frequently to secondary calcification. Some tubules showed a flat regenerating epithelium and occasionally mitotic figures. Focal areas of chronic inflammation were fairly prominent. In all cases, some of the cortical tubules contained scattered brown-staining granules similar to those previously described by Fairhill and Miller.[8] In addition, occasional round, concentric bodies were noticed most often lying within the lumen of the tubules. These bodies stained brownish and occasionally somewhat bluish in hematoxylin-eosin preparations. Many of these bodies stained black after treatment of the sections with cobalt chloride and dilute ammonium sulphide, indicating the presence of calcium phosphate. They obviously represented partially calcified necrotic cells. Control sections immersed in dilute ammonium sulphide did not show any blackening of these bodies or of the intranuclear inclusions or of any other structures. The absence of histochemically demonstrable lead is in agreement with Blackman's finding but contrary to those of Finner and Calvery. Basophilic cytoplasmic inclusion bodies described by Diaz-Rivera and Horn were not seen in our material.

In sections stained by the Ziehl-Neelsen method, the inclusion bodies were strongly acid-fast and stained red (Fig. 1). There was some variation in the brilliance of the stain even in sections of the same block. Occasionally inclusion bodies took a more purplish hue after the counterstain had been applied. This staining method revealed fine dust-like acid-fast particles in some of the nuclei. The counterstain clearly outlined the chromatin material surrounding the inclusion bodies within the nuclei. In addition, some of the tubules contained dust-like and occasionally more granular acid-fast material. This apparently corresponded to the previously described brown pigment seen in hematoxylin and eosin sections, but seemed to be more abundant.

Two unstained paraffin sections from human material that had been collected by the late Dr. S. Blackman were obtained through the courtesy of Dr. Lucas. The inclusion bodies proved to be acid-fast although they stained somewhat less brilliantly than most sections from the fresh animal material. In the control animals (fed nickel or cobalt) no acid-fast material was encountered. No inclusion bodies or any of the other significant changes were found in the kidneys of any of the control animals fed either cobalt or nickel.

Bismuth. The cells of the convoluted tubules show inclusion bodies as described by Pappenheimer and Maechling[25] within the nuclei and occasionally also in the cytoplasm. These inclusion bodies were strongly refractile and in hematoxylin and eosin sections had a brownish tinge. They were usually round, occa-

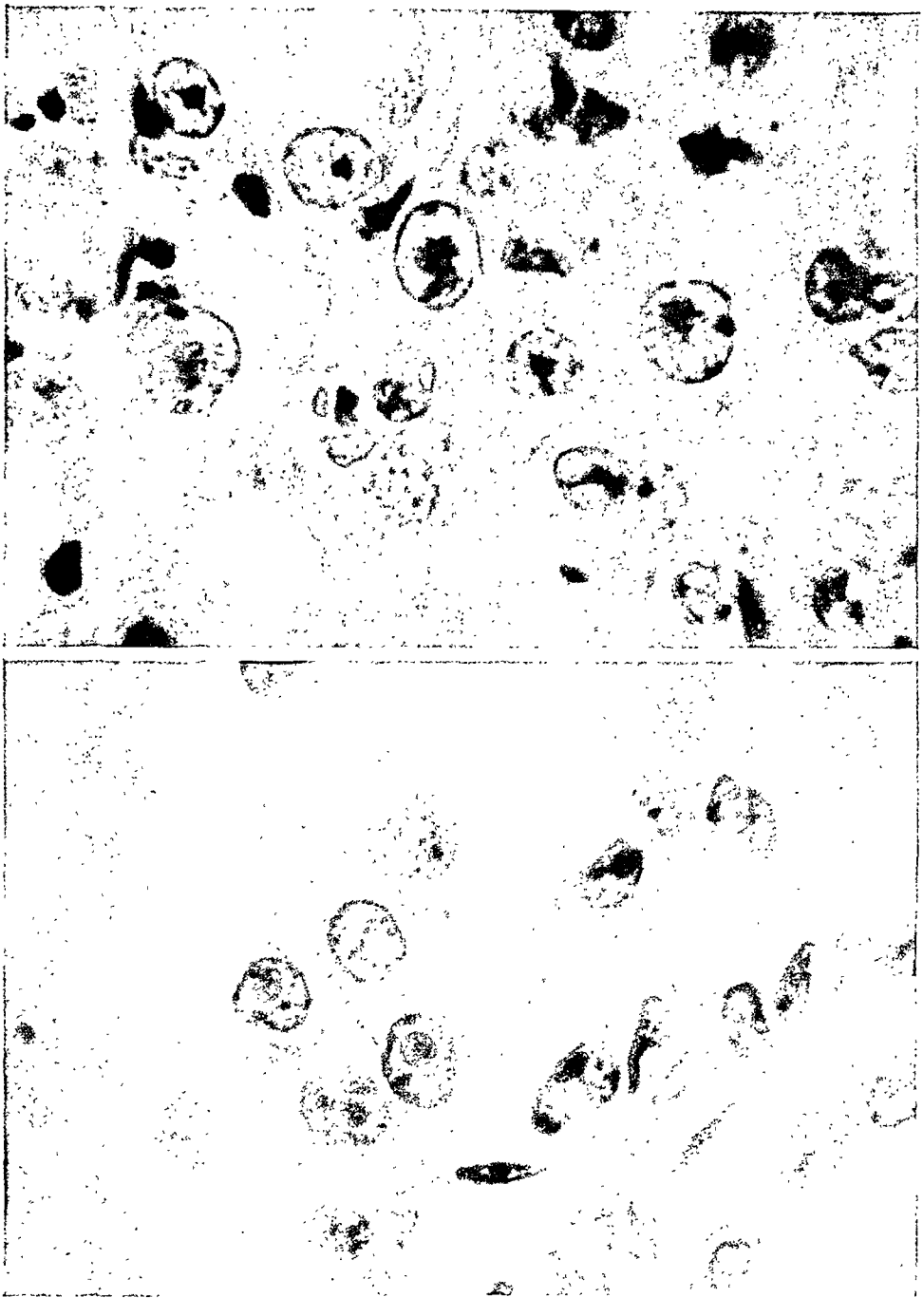


FIG. 1. Section from the kidney of a rat which was given lead acetate. Ziehl-Neelsen stain. Nuclear inclusion bodies stain brilliantly red. Approximately × 900.

FIG. 2. Section from the kidney of a woman who had received weekly bismuth injections for one year. Ziehl-Neelsen stain. Nuclear inclusion bodies stain brilliantly red. Approximately × 900.

sionally oval, and often occupied a central position in the nucleus. Some of the nuclei contained several of these inclusion bodies. Degenerative changes in the tubular epithelium were difficult to evaluate since the kidney, as previously stated, was obtained from a patient who had had malignant hypertension.

The application of the Ziehl-Neelsen stain revealed that the inclusions were strongly acid-fast. Both intranuclear and cytoplasmic inclusion bodies stained a brilliant red (Fig. 2). No other structures were found to retain the carbol fuchsin after acid-alcohol treatment. The smallest inclusions could be easily visualized by this staining method.

Other inclusion bodies. None of the other nuclear inclusion bodies that were examined were acid-fast. These included the nuclear inclusion bodies present in the salivary glands of a child, as well as those found in liver and kidneys of a newborn infant; the numerous inclusion bodies present in the alveolar lining cells of an adult woman who died of pneumonitis; the nuclear inclusion bodies in the beta cells of the pancreas of the chicken, recently described by Lucas;[16] Negri bodies; the inclusions in distemper, and in the intestinal epithelium of suckling mice with diarrhea described by Pappenheimer and Cheever;[24] or the inclusion bodies caused by the Virus III.

DISCUSSION

Nuclear inclusion bodies have been divided by Cowdry[3] into types A and B. Type A included those which are granular, while type B consist of amorphous, irregular, masses or hyaline spheres. However, in the study of inclusion bodies, it is not only necessary to recognize their existence, but also to separate them from changes that may be caused by the damaging agent itself, on the cellular constituents. By studying their development, from the earliest appearance to the final stage when the cell dies, one is able to recognize distinct differences in various inclusion bodies, as has been clearly shown by Lucas. On the basis of studies of this kind, it seems that in severe burns, yellow fever, and in panleukopenia of the cat, the inclusion bodies are derived from the oxychromatin which is normally present in the nucleus but has been increased in amount.[18] In fox encephalitis, the acidophilic portion of the inclusion bodies seems to come from the oxychromatin surrounding the primary basophilic center.[14] On the other hand, in the case of inclusion bodies in the beta cells of the islands of Langerhans in chickens, there is no proof for a derivation of inclusion bodies from oxychromatin. In the submaxillary gland virus, there is no definite morphologic evidence that the acidophilic part is derived from chromatin.[28] There is considerable difference of opinion as to the origin of the inclusions in the herpes, B. virus and pseudorabies group. While Luger and Lauda[19] considered the inclusion bodies of herpes as accumulated masses of oxychromatin, this is questioned by Lucas and Herrmann.[17]

In contrast, the inclusion bodies produced by aluminum oxide[1] and also those in lead poisoning[13] are supposed to be derived from the plasmosome portion of an amphinucleolus. It seems of considerable interest that of all inclusion bodies examined only those caused by ingestion of metals proved to be acid-fast. If, however, these inclusions were derived from the acidophilic portion of the nu-

cleolus, a considerable change must have taken place, since the nucleolus itself is not acid-fast.

The acid-fastness of these inclusion bodies raises the question of the possible chemical significance of this staining reaction. It is known that certain cell constituents may show acid-fastness. A coarsely globular wax-like pigment is found in the cirrhotic livers as well as other tissues of rats maintained on a low protein choline-deficient diet. It was called ceroid.[14] Its deposition is strongly influenced by the amount of cod liver oil given in the diet.[29] A similar pigment occurs also in the smooth muscle cells of the uterus and in other tissues in rats on a diet deficient in vitamin E.[20, 21] In certain nutritional disorders and hepatic diseases in man, Pappenheimer and Victor[26] found similar acid-fast pigment in various tissues. Acid-fastness has been reported in the lipoid deposits in the lungs with cod liver oil pneumonia.[10, 27] Endicott[7] was able to show that prolonged oxidation of cod liver oil or a linseed oil *in vitro* with potassium bichromate renders these oils acid-fast. Linseed and cod liver oil injected into various tissues of living rats acquire acid-fastness.[7, 11]

The acid-fastness of bacteria and animal tissue is apparently of a variegated and complex nature. In tubercle bacilli there exists a hydroxy acid, mycolic acid isolated from the unsaponifiable wax which exhibitis acid-fastness even after its isolation as a pure substance. On the other hand, acid-fastness in tubercle bacilli is destroyed after the disintegration of the cellular structure by various means which leave the mycolic acid intact. Endospores of bacteria, as well as human hair retain carbol fuchsin although these substances do not contain large amounts of lipoids or mycolic acid.

It may be concluded that some lipoid component is responsible for the acid-fastness of certain inclusion bodies. Acid-fastness, however, is not the unique attribute of a substance or a group of substances, but rather is the sign of some peculiarity of cellular organization related, for instance, to the physicochemical property of the cell wall (Dubos[6]).

A certain relationship of some inclusion bodies to lipoid has already been suggested by the finding of Pappenheimer and Maechling that bismuth-induced inclusions can be demonstrated by the Weigert-Spielmayer stain for myelin. In contrast, however, to the behavior of typical ceroid, the inclusion bodies do not stain with the usual fat stains, as has already been demonstrated for lead by Blackman and for bismuth by Pappenheimer and Maechling.

SUMMARY

Various types of inclusion bodies were examined with the Ziehl-Neelsen technic for acid-fastness. Among those examined, only inclusion bodies produced by the ingestion of bismuth and lead were found to be acid-fast. Aside from the possible significance as to their lipoid content, the Ziehl-Neelsen stain is a convenient method for the easy demonstration of these inclusion bodies.

REFERENCES

1. Birch, F. M., and Lucas, A. M.: Effects of centrifugation on intranuclear inclusions produced by subcutaneous injections of aluminum oxide. Am. J. Path., **18**: 1051–1059, 1942.
2. Blackman, S. S., Jr.: Intranuclear inclusion bodies in the kidney and liver caused by lead poisoning. Bull. Johns Hopkins Hosp., **58**: 384–404, 1936.

3. COWDRY, E. V.: The problem of intranuclear inclusions in virus diseases. Arch. Path., **18:** 527–542, 1934.
4. DALLDORF, G., AND WILLIAMS, R. R.: Impairment of reproduction in rats by ingestion of lead. Science, **102:** 668–670, 1945.
5. DIAZ-RIVERA, R. S. AND HORN, R. C., JR.: Postmortem studies on hypertensive rats chronically intoxicated with lead acetate. Proc. Soc. Exper. Biol. and Med., **59:** 161–163, 1945.
6. DUBOS, R. J.: The bacterial cell in its relation to problems of virulence, immunity and chemotherapy. Cambridge: Harvard University Press, pp. 85–91, 1947.
7. ENDICOTT, K. M.: Similarity of the acid-fast pigment ceroid and oxidized unsaturated fat. Arch. Path., **37:** 49–53, 1944.
8. FAIRHALL, L. T., AND MILLER, J. W.: The deposition and removal of lead in the soft tissues (liver, kidneys and spleen). Pub. Health Rep., **56:** 1641–1650, 1941.
9. FINNER, L. L., AND CALVERY, H. O.: Pathologic changes in rats and in dogs fed diets containing lead and arsenic compounds; compounds used: lead arsenate, arsenic trioxide, calcium arsenate and lead acetate. Arch. Path., **27:** 433–446, 1939.
10. GRAEF, I.: Studies in lipid pneumonia; lipid pneumonia due to cod liver oil; lipid pneumonia due to liquid petrolatum. Arch. Path., **28:** 613–667, 1939.
11. HAAS, G. M.: Membrane formation at lipoid-aqueous interfaces in tissues; a correlation of the morphological and chemical aspects. Arch. Path., **28:** 177–198, 1939.
12. LILLIE, R. D., ASHBURN, L. L., SEBRELL, W. H., JR., DAFT, F. S., AND LOWRY, J. V.: Histogenesis and repair of the hepatic cirrhosis in rats produced on low protein diets and preventable with choline. Pub. Health Rep., **57:** 592–608, 1942.
13. LUCAS, A. M.: Personal communication to the author.
14. LUCAS, A. M.: The cytology of fox encephalitis and the effects of centrifugation upon the intranuclear inclusions. Am. J. Path., **16:** 739–760, 1940.
15. LUCAS, A. M.: Inclusion bodies. How specific are inclusion bodies for the identification of virus diseases? The Veterinary Student, **4:** 11–14 and 45–46, 1941.
16. LUCAS, A. M.: Intranuclear inclusions in the islands of Langerhans of chickens. Am. J. Path., **23:** 1005–1022, 1947.
17. LUCAS, A. M., AND HERRMANN, W. W.: Effect of centrifugation on herpetic intranuclear inclusions with a note on cytoplasmic inclusions of unknown origin in the rabbit cornea. Am. J. Path., **11:** 963–976, 1935.
18. LUCAS, A. M., AND RISER, W. H.: Intranuclear inclusions in panleukopenia of cats; correlation with the pathogenesis of the disease and comparison with inclusions of herpes, B-virus, yellow fever, and burns. Am. J. Path., **21:** 435–465, 1945.
19. LUGER, A., AND LAUDA, E.: Über oxychromatische Veränderungen am Zellkern. (Auf Grund von Untersuchungen von Herpes simplex, zoster, Varizellen, Variola und Karpfenpocke.) Ein Beitrag zur Kenntnis und Wertung einschlussartiger Gebilde. Med. Klin., **22:** 415–417; 456–458; 493–497, 1926.
20. MASSON, K. E., AND EMMEL, A. F.: Vitamin E and muscle pigment in the rat. Anat. Rec., **92:** 33–59, 1945.
21. MASSON, K. E., AND EMMEL, A. F.: Pigmentation of the sex glands in Vitamin E deficient rats. Yale J. Biol. and Med., **45:** 189–202, 1944.
22. OLITSKY, P. K., AND HARFORD, C. G.: Intranuclear inclusion bodies in the tissue reactions produced by injections of certain foreign substances. Am. J. Path., **13:** 729–748, 1937.
23. OLIVER, J.: In Harvey Lectures. Vol. 40. Lancaster, Pa.: Science Press Printing Company, p. 102, 1944–1945.
24. PAPPENHEIMER, A. M., AND CHEEVER, F. S.: Epidemic diarrheal disease of suckling mice; cytoplasmic inclusion bodies in intestinal epithelium in relation to the disease. J. Exper. Med., **88:** 317–324, 1948.
25. PAPPENHEIMER, A. M., AND MAECHLING, E. H.: Inclusions in renal epithelial cells following the use of certain bismuth preparations. Am. J. Path., **10:** 577–588, 1934.
26. PAPPENHEIMER, A. M., AND VICTOR, J.: "Ceroid" pigment in human tissues. Am. J. Path., **22:** 395–413, 1946.
27. PINKERTON, H.: The reaction to oils and fats in the lung. Arch. Path., **5:** 380–401, 1928.
28. ROSENBUSCH, C. T., AND LUCAS, A. M.: Studies in the pathogenicity and cytological reactions of the submaxillary gland virus of the guinea pig. Am. J. Path., **15:** 303–340, 1939.
29. WACHSTEIN, M.: Influence of cod liver oil on deposition of ceroid in the nutritional rat cirrhosis. Proc. Soc. Exper. Biol. and Med., **59:** 73–77, 1945.
30. WACHSTEIN, M.: Nephrotoxic action of *dl*-serine in the rat; localization of the renal damage, the phosphatase activity and the influence of age, sex, time and dose. Arch. Path., **43:** 503–514, 1947.
31. WACHSTEIN, M., AND ZAK, F. G.: Bismuth pigmentation; its histochemical identification. Am. J. Path., **22:** 603–611, 1946.

LECITHINASE ACTIVITY IN SPLENIC DYSCRASIAS*

GEORGE J. SCHEFF, M.D., AND AHMED J. AWNY, M.D.,† (ALEXANDRIA, EGYPT)

From the Departments of Medical Research and Pathology, Ohio State University, Columbus, Ohio

Red blood cells are constantly destroyed *in vivo*. The mechanism of this process is extremely complex and at present only incompletely understood. While phagocytosis and fragmentation (Rous[21]) may sufficiently explain the normal destruction of the blood corpuscles, in certain anemias lytic factors become preponderant and their action results in pathologic conditions.

It has been stated that excessive hemolysis is due either to normal hemolytic agents acting on defective erythrocytes, or to the exposure of red blood cells of normal resistance to lytic agents in unusual quantity or of unusual activity.[18] Whichever the case may be, the spleen must play an important role, since splenectomy has proved to be a life-saving operation in the clinical management of certain hemolytic syndromes. Evidence seems to accumulate that the macrophages (phagocytic clasmatocytes) of the spleen are specifically responsible for the abnormal activity. They not only dispose of the effete blood cells by engulfing them, but also elaborate various enzymes which prepare the blood cells for destruction. The unique circulatory conditions in the spleen are especially favorable for this latter function.

The present paper deals with the observation that in blood dyscrasias with excessive hemolysis, the lecithinase activity of the spleen is increased; this in turn can be demonstrated by the enhanced lytic activity of lysolecithin.

METHODS

To assure comparable results a standardized procedure was adopted. The spleens were obtained from persons immediately following operation, or shortly after death. They were trimmed of fat and connective tissue, weighed and thoroughly minced in a meat grinder. Portions (10 Gm.) of this mince were weighed into centrifuge tubes, then washed 3 times with sterile saline and once with phosphate buffer (pH 7.1) by slow centrifugation. This freed the tissues from blood while the heavy particles, including the fixed cells, collected at the bottom of the tubes; the washing fluids were discarded.

Each test for lecithinase activity consisted of a set of 3 or 4 samples containing: (a) 10 Gm. of washed tissue mince and 25 ml. lecithin emulsion in buffer (as substrate); (b) 10 Gm. of tissue mince suspended in 25 ml. of buffer; (c) same as (a), but inactivated in boiling water bath for 15 minutes; (d) 25 ml. of lecithin emulsion in buffer. The substrate was prepared by emulsifying one fresh egg yolk in 100 ml. of Sörensen's phosphate buffer (pH 7.1), using a Waring blender. (Purified egg lecithin can also be used, but was found to be less stable in emulsion.) This buffer (pH 7.1) was selected as optimum for lecithinase activity;[5] the amount was sufficient to balance any changes caused by tissue autolysis. Contamination was prevented by adding to each sample a few drops of chloroform and raising the incubation temperature to 43°C. An arbitrary incubation period of 48 hours was applied to all samples following which all were filtered and the residues washed with a

* Received for publication, April 8, 1949.

† Dr. Awny was aided by a grant from the Egyptian Government.

minimal amount (5 ml.) of buffer solution. Then the filtrates (about 30 ml.) were precipitated with 10 times their volume (about 300 ml.) of absolute ethyl alcohol and refiltered. The alcoholic extracts were evaporated under reduced pressure to dryness and the resulting residues redissolved in 15 ml. alcohol. The undissolved particles of each sample were filtered off and the filtrates (in large 250 ml. centrifuge tubes) precipitated by adding 6 times their volume of ether. The tubes containing the mixture were stoppered and allowed to stand overnight in the cold room. The following day the ether-insoluble precipitate was centrifuged, the liquid decanted and the remaining traces of ether expelled by a current of nitrogen. Finally, the practically dry precipitate was dissolved in 2 ml. of saline, then briefly spun off and the supernatant used for testing the hemolytic activity.

Among the various proposed procedures the dilution method and the visual observation of hemolysis seemed to be the most practical. The dilutions covered a range of 1:2 to 1:1024 in geometric progression, each tube containing a total volume of 1 ml. The corpuscles used for testing were normal red cells which were washed 3 times with saline and made up in a 2 per cent suspension. One drop of this suspension was added to each tube, including the control. The tests were carried out at room temperature and were read after two hours.

RESULTS

Over a period of one year (1946–47) 10 pathologic and 2 normal spleens were studied. The splenectomies were performed either as an emergency measure (by Dr. G. M. Curtis or Dr. R. M. Zollinger), or in cases of chronic debility after consultation with members of the Department of Medicine (Service of Dr. C. A. Doan or Dr. B. K. Wiseman). The 2 normal spleens and one from a patient with polycythemia vera were recovered from autopsies (through the courtesy of Dr. E. von Haam) not later than six hours after death.

The patients that were operated upon had diseases that were typical of essential thrombocytopenic purpura, splenic neutropenia and congenital hemolytic icterus. In all of them the spleen was the major factor in their ailment, so that the term, "hypersplenism" was applicable to all of them.[10]

Table 1 lists the results of the completed hemolytic tests as they were read at the end of the second hour. As can be seen, there is quite a difference between the hemolytic activity of the normal and pathologic spleen. Whereas the addition of lecithin to the splenic tissue did not alter the hemolytic titer of the normal spleens, the same procedure always raised the titer 4 to 256 times in the hypersplenic ones. The extent of increase seems to parallel the acuity of the hemolytic process in the patient before the operation. In chronic cases the increase was usually modest, while in the acute phases of these diseases which required emergency operations, it was, as a rule, greatly enhanced. The observed changes in enzymatic activities are indicated in the table. The figures in the last column represent the ratios between the titers of the "a" and "b" series. The highest titer was found in a polycythemic spleen. Although this disease should not be classed with the other types of hypersplenism, it appears that in this disease an increased hemolytic process is invoked, and maintained, to compensate for the overproduction of red blood cells. Furthermore, the tests with this spleen supported the validity of our findings with the control spleens since the organ was obtained from an autopsy, and was not extirpated. No traumatic spleens were available at the time of these studies.

DISCUSSION

The preceding experiments demonstrated that the lytic potency of hyperactive spleens is increased. This finding may be supplemented by the following comments:

TABLE 1

HEMOLYTIC TITERS OF LYSOLECITHIN EXTRACTED FROM NORMAL AND ABNORMAL SPLEENS

NO. OF CASE	PATIENT	DIAGNOSIS	TYPE OF EXPERIMENT		HEMOLYTIC TITER OF EXTRACTED LYSOLECITHIN†										C	RATIO a:b
			Series*	Components**	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024		
1	M	Thrombocytopenic purpura	a	S + L + B	+++	+++	++	+	0						0	4
			b	S + B	+++	+	0								0	
2	Cr	Thrombocytopenic purpura	a	S + L + B	++	++	+	0							0	4
			b	S + B	++	0									0	
3	R	Thrombocytopenic purpura	a	S + L + B	+++	+++	+++	+++	+	0					0	16
			b	S + B	+	0									0	
4	S	Splenic neutropenia	a	S + L + B	+++	+++	+++	+++	++	+	0				0	32
			b	S + B	++	0									0	
5	D	Splenic neutropenia	a	S + L + B	+++	+++	+++	++	+	0					0	8
			b	S + B	++	+	0								0	
6	C	Splenic neutropenia	a	S + L + B	+++	+++	+++	+++	+++	+++	+++	+++	+	0	0	64
			b	S + B	+++	++	+	0							0	
7	L	Hypersplenism (?)	a	S + L + B	+++	+++	+++	+++	+++	+++	+++	+	0		0	64
			b	S + B	++	+	0								0	
8		Polycythemia vera	a	S + L + B	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	0	256
			b	S + B	++	+	0								0	
9	E	Congenital hemolytic icterus	a	S + L + B	+++	+++	+++	+++	+++	+++	0				0	16
			b	S + B	++	+	0								0	
10	Y	Congenital hemolytic icterus	a	S + L + B	+++	+++	+++	+++	+++	+++	++	+	0		0	128
			b	S + B	+	0									0	
11	Co	Congenital hemolytic icterus	a	S + L + B	+++	+++	+++	+++	++	+	0				0	16
			b	S + B	+++	+	0								0	
12		Normal	a	S + L + B	++	+	0								0	2
			b	S + B	+	0									0	
13		Normal	a	S + L + B	+	0									0	1
			b	S + B	+	0									0	

* Each experiment included two additional series of controls: Series c (lecithin and buffer) and Series d (splenic tissue, lecithin and buffer heated to 100 C. for 15 minutes). They remained constantly negative and hence were omitted from the table.

** S indicates washed splenic tissue; L, lecithin emulsion; B, buffer (pH 7.1); and C, control.

† + indicates slight hemolysis; ++, partial hemolysis; and +++, complete hemolysis.

First, it should be pointed out that the discovery of erythrolytic properties in tissues, and their extracts (not restricted to the spleen alone) is not entirely new. As early as the first decade of this century, substantial contributions to

this subject had already been made (see Krumbhaar[14] and Lauda[15]). In these studies, specific as well as nonspecific factors had been proposed in an attempt to explain hemolysis *in vivo*. It seems, however, that there is but little evidence for demonstrating the lytic destruction of normal erythrocytes whereas the evidence is strong[20] in favor of the predominant role of hemolysis under certain pathologic conditions.

Second, not only one but several factors may be involved in producing hemolysis *in vivo*. This is particularly important in explaining the involvement of the spleen (a) by actively destroying the red blood cells; (b) by modifying the red corpuscles so as to prepare them for later destruction; (c) by elaborating specific, or nonspecific hemolytic substances. Lysolecithin would belong to this latter category.

Assuming that the same mechanism may be effective as the one originally proposed for cobra venom hemolysis, Belfanti[1] and his associates[5] were among the first to demonstrate the formation of lysolecithin as the result of lecithinase activity in tissues. This finding was corroborated by others, but it was not until Bergenhem and Fähraeus[2] re-introduced this concept that it was related to hemolytic processes *in vivo*. Accordingly, the red cells would adsorb lysolecithin, and by becoming spherical, would be sensitized to subsequent lysis. Several outstanding investigators (Collier and Wilbur,[4] Dameshek and Schwartz,[6] Dameshek, Schwartz and Singer,[7] Foy and Kondi,[8] Gripwall,[9] Lloyd,[16] Singer[22, 23, 24] supported this working hypothesis, but limited their studies to the demonstration of lysolecithin in the blood. Since plasma, even in high dilutions, proved to be inhibitory to lysolecithin activity, many objections were raised which serve to delay the general acceptance of this concept.

Recently Maegraith and his associates[17] took up this subject again. They demonstrated the presence of a specific hemolytic enzyme (erythrocytase) by incubating washed tissue slices with washed red cell suspension. Their main points in favor of a lytic enzyme have been the demonstration of species specificity, inhibition by enzyme poisons and inactivation by heat. Ponder[20] in his excellent paper confirmed most of their findings (except the species specificity) and advanced the view that lecithinase (lysolecithinase?) might be the enzyme responsible for the liberation of the lytic substance and that the agent itself is lysolecithin. The latter is probably a normal intravascular lysin widely produced by the tissues (not in the spleen alone) and its lytic activity in the blood probably depends on the concentration and effectiveness of the inhibitors. Hence, an unbalanced reduction of inhibition would result in abnormally increased red cell destruction. Contrary to these observations, Brückmann and Wertheimer[3] failed to extract any lysolecithin from fresh, or even autolyzed tissues; moreover, they found no increase in lytic activity on addition of lecithin. They expressed, therefore, serious doubts concerning the significance of lecithinase in blood destruction and assumed that autolytic products (fatty acids) are more likely to be responsible for the resulting hemolysis (see also Ham and Castle[11]; Tsai, Chen and Chiu[25]).

In view of these opposing results, it was interesting to find that in our own experiments the addition of lecithin increased the hemolytic activity of hyper-

active spleens only. Furthermore, adequate controls proved that the process is enzymatic in nature and that autolysis plays a negligible part. This is quite consistent with the observation of Hicks and Opie[12] who demonstrated an increase in enzymatic proteolysis in rabbit spleens with enhanced phagocytic activity.

Finally, it would seem that the key position of the spleen in the hemolytic processes deserves an explanation. The mere demonstration that the reticuloendothelial cells of the spleen are numerically increased and functionally hyperactive in producing the enzyme would not be sufficient to explain the whole picture. These cells are known to be widely distributed in the organism and are not restricted to the spleen alone. Still, we know that after splenectomy the red cell destruction is completely halted in most of the cases. Hence, in the spleen special conditions must prevail which specially contribute to the lytic process.

As previously shown by others,[11, 22, 25] blood stagnation is one of the prerequisites for the action of lysolecithin. The spleen is the only organ which can provide the necessary conditions for this effect. In the spleen, according to McNee[19] and Knisely,[13] the circulation is largely cut off from the main blood flow and will permit the blood to stagnate for various lengths of time. A complete separation between the cells and plasma has also been claimed by some investigators. Under these circumstances the lecithinase activity will not only be enhanced in hypersplenism due to the increased number of macrophages in the spleen but simultaneously the inhibitory effect of plasma will be reduced to a minimum. In addition, a more intimate contact between the blood cells retained in the meshwork of the pulp and the freshly elaborated lysolecithin can be established. This may gradually lead to spherocytosis and to sensitization of the erythrocytes for hemolysis. Following splenectomy the spherocytosis may still persist because of continued formation of lysolecithin in other parts of the body, but in the absence of the spleen the principal site of destruction is eliminated. The fundamental difference in the action of lysolecithin in stagnant and circulating blood is the best explanation for its striking action in the hyperactive spleen.

We find in accordance with this theory the fact that the lysolecithin activity is not necessarily confined to the destruction of the red blood cells alone but is equally effective against all formed blood elements.[1] It will act primarily on those blood cells which have been retained in the splenic pulp, thereby varying with the nature of the blood dyscrasia.

SUMMARY

In spleens from patients suffering with thrombocytopenic purpura, splenic neutropenia and congenital hemolytic icterus an increased lecithinase activity could be demonstrated by increased formation of lysolecithin *in vitro*. In two normal spleens obtained at autopsy, the same effect was not present.

The significance and possible implications of these findings in hypersplenism are discussed.

REFERENCES

1. BELFANTI, S.: Über das Hämoleukolysin des Pankreas und dessen Bezug zum Delezenne-Fourneauschen Lysocithin. Biochem. Ztschr., **154:** 148–166, 1924.
2. BERGENHEM, B., AND FÄHRAEUS, R.: Über spontane Hämolysinbildung im Blut, unter besonderer Berücksichtigung der Physiologie der Milz. Ztschr. f. d. ges. exper. Med., **97:** 555–587, 1936.
3. BRÜCKMANN, G., AND WERTHEIMER, E.: Lysis of red blood cells by tissue slices. Brit. J. Exper. Path., **26:** 217–224, 1945.
4. COLLIER, H. B., AND WILBUR, K. M.: Lysolecithin and the antihemolytic value of the blood. J. Lab. and Clin. Med., **29:** 1123–1133, 1944.
5. CONTARDI, A., AND ERCOLI, A.: Über die enzymatische Spaltung der Lecithine und Lysolecithine. Biochem. Ztschr., **261:** 275–302, 1933.
6. DAMESHEK, W., AND SCHWARTZ, S. O.: Hemolysins as the cause of clinical and experimental hemolytic anemias. Am. J. M. Sc., **196:** 769–792, 1938.
7. DAMESHEK, W., SCHWARTZ, S. O., AND SINGER, K.: Spherocytosis and increased erythrocyte fragility as indicators of hemolytic activity. J. Clin. Investigation, **18:** 479, 1939.
8. FOY, H., AND KONDI, A.: Lyso-lecithin fragility in black-water fever and haemolytic jaundice. Tr. Roy. Soc. Trop. Med. and Hyg., **37:** 1–18, 1943.
9. GRIPWALL, E.: Zur Klinik und Pathologie des hereditären hämolytischen Ikterus. Acta med. Scandinav., Suppl. **96:** 1–290, 1938.
10. VON HAAM, E., AND AWNY, A. J.: The pathology of hypersplenism. Am. J. Clin. Path., **18:** 313–322, 1948.
11. HAM, T. H., AND CASTLE, W. B.: Relation of increased hypotonic fragility and of erythrostasis to the mechanism of hemolysis in certain anemias. Tr. A. Am. Physicians, **55:** 127–132, 1940.
12. HICKS, S. P., AND OPIE, E. L.: Proteolytic digestion of red and white blood corpuscles in the spleen. Am. J. Path., **18:** 333–341, 1942.
13. KNISELY, M. H.: Spleen studies; microscopic observations of the circulating system of living unstimulated mammalian spleens. Anat. Rec., **65:** 23–50, 1936.
14. KRUMBHAAR, E. B.: Functions of the spleen. Physiol. Rev., **6:** 160–200, 1926.
15. LAUDA, E.: Die normale und pathologische Physiologie der Milz. Berlin: Urban, 280 pp., 1933.
16. LLOYD, T. W.: Thesis, Oxford Univ., 1940.
17. MAEGRAITH, B. G., MARTIN, N. H., AND FINLAY, G. M.: The mechanism of red cell destruction. Brit. J. Exper. Path., **24:** 58–65, 1943.
18. MAIZELS, M.: The action of haemolysins on normal and pathological human erythrocytes. Quart. J. Exper. Physiol., **33:** 183–214, 1946.
19. MCNEE, J. W.: The spleen; its structure, functions and diseases. Lancet, **220[1]:** 951–957; 1009–1014; 1063–1070, 1931.
20. PONDER, E.: The mechanism of the inhibition of hemolysis. J. Gen. Physiol., **27:** 1–14, 1943.
21. ROUS, P.: Destruction of the red blood corpuscles in health and disease. Physiol. Rev., **3:** 75–105, 1923.
22. SINGER, K.: Lysolecithin and hemolytic anemia; significance of lysolecithin production in the differentiation of circulating and stagnant blood. J. Clin. Investigation, **20:** 153–160, 1941.
23. SINGER, K., MILLER, E. B., AND DAMESHEK, W.: Hematologic changes following splenectomy in man with particular reference to target cells, hemolytic index and lysolecithin. Am. J. M. Sc., **202:** 171–187, 1941.
24. SINGER, K., AND WEISZ, L.: The life cycle of the erythrocyte after splenectomy and the problem of splenic hemolysis and target cell formation. Am. J. M. Sc., **210:** 301–323, 1945.
25. TSAI, C., CHEN, C. J., AND CHIU, K. Y.: Observations on the various factors influencing the increase of erythrocytic fragility induced by stasis. Am. J. Physiol., **138:** 519–526, 1943.

STUDIES ON THE BLOOD FACTOR rh^w*

ALEXANDER S. WIENER, M.D., AND EVE B. GORDON

From the Serological Laboratory, Office of the Chief Medical Examiner of New York, New York

In 1945, Callender, Race and Paykoc[3] reported a remarkable case in which a patient with diffuse lupus erythematosus treated by repeated blood transfusions became isosensitized to 5 agglutinogens in succession, namely, the agglutinogens hr′ and N, and 3 others previously undescribed. The purpose of this paper is to describe some studies on one of the new blood factors discovered by these workers, namely, the factor designated C^w by them, after the donor Willis whose blood stimulated the production of the specific antibody.

In their initial study of 768 blood specimens, Callender and Race[2] encountered 19 bloods which gave positive reactions with the anti-Willis serum. All the positive-reacting bloods possessed the factor rh′, indicating that a relationship existed between the Willis factor and the rh′ factor. Since the rh′ factor is designated as C under the British notations, the new factor was given the designation C^w. Under the International Rh-Hr Nomenclature, type Rh$_1$ blood containing the Willis factor was designated as Rh$_1^w$, and the corresponding gene was designated R^{1w}. The work of Callender and Race was soon confirmed when other instances of sensitization to the Willis factor were encountered by Lawler and van Loghem,[8] and by Broman.[1]

The main purpose of the present paper, aside from presenting our own observations on the Willis factor, is to attempt to clarify the relationship of the new blood factor and antiserum to the previously found Rh factors. For reasons which will become apparent, in the present paper the Willis factor will be designated as the rh^w factor.

MATERIALS AND METHODS

An active sample of anti-rh^w serum was received from Dr. R. R. Race, but after some 40 or 50 tests had been made the supply of this serum was exhausted. Another sample of anti-rh^w was sent by Dr. J. J. van Loghem but this was inactive when received and could not be used. The bulk of the work described in this paper was carried out with potent anti-rh^w agglutinating serum kindly supplied by Dr. Birger Broman.

In view of the scarcity of the anti-rh^w serum, the following technic was evolved. The tests were set up by the usual agglutination method including an rh^w-positive control, but in small tubes having an inside diameter of 4 to 5 mm. instead of the usual tubes with a diameter of 7 to 8 mm. In this way smaller drops of serum and of cell suspensions could be used. After the tests were completed and the readings taken in the usual manner, the few tubes showing positive reactions were discarded. From the remaining tubes, the anti-rh^w serum was pipetted off in order to be used again in further tests. This process could usually be repeated as many as 5 or 6 times before the anti-rh^w serum had to be discarded. In this way 1 ml. of reagent could be used for as many as 200 tests. Since with succeeding tests, the serum becomes more and more diluted with the saline from the red cell suspensions, it is necessary to increase correspondingly the volume of diluted serum used in such

* Received for publication, March 12, 1949.

tests. Thus, the first time the reagent is used only one drop of antiserum is used per test; the second time, 2 drops of serum is used per test; the third time, 3 drops; etc. This process may be continued indefinitely, as long as the positive control shows satisfactory reactions and the serum remains uncontaminated. When the serum becomes more highly diluted we find it convenient, after the cells have sedimented and before the readings are taken, to remove the supernatant and substitute a single drop of saline. We have applied this economical technic also to other rare antiserums giving a low per cent of positive reactions, such as anti-rh″ serum and anti-Si serum.[18]

All blood specimens were also tested for the A-B-O blood groups, the M-N factors and the Rh-Hr types in the usual manner. For the Rh-Hr typings, all the reagents used were of the agglutinating variety. The anti-Rh_0 serum was derived from a mother of an erythroblastotic baby who had been stimulated by an injection of Rh-positive blood. Two anti-rh′ reagents were available, both prepared from anti-Rh_0' serums to which had been added

TABLE 1

INCIDENCE OF THE rh^w FACTOR AMONG CAUCASOIDS, NEGROIDS AND CHINESE

Rh-Hr TYPE	CAUCASOIDS			NEGROIDS			CHINESE		
	Positive	Negative	Totals	Positive	Negative	Totals	Positive	Negative	Totals
rh	0	38	38	0	6	6	0	0	0
Rh_1rh	4	62	66	0	30*	30	0	5	5
Rh_1Rh_1	3	55	58	0	3	3	0	12	12
Rh_2rh	0	29	29	0	16	16	0	2	2
Rh_2Rh_2	0	5	5	0	2	2	0	1	1
Rh_zRh_0**	3	42	45	0	2	2	0	10	10
Rh_0	0	8	8	0	42	42	0	1	1
rh′rh	0	5	5	0	3	3	0	0	0
rh″rh	0	5	5	0	0	0	0	0	0
Totals.....	10	249	259	0	104	104	0	31	31

* Includes 4 bloods giving "intermediate" reactions with anti-rh′ serum.

** Type Rh_zRh_0 blood belongs to type Rh_1Rh_2 and is both hr′-positive and hr″-positive.

potent anti-Rh_0 blocking serum. Two anti-rh″ serums were available, one a pure anti-rh″ agglutinating serum obtained from a type Rh_1 mother of an erythroblastotic baby, and the other prepared from an anti-Rh_0'' serum by the addition of Rh_0 blocking serum. The anti-hr′ serum was obtained from a type Rh_1Rh_1 mother of an erythroblastotic baby, while the anti-hr″ serum was obtained from a type Rh_2Rh_2 individual who had become sensitized after multiple transfusions and pregnancies.

The bloods tested were derived from persons of Caucasoid, Negroid and Chinese origins. The Caucasoid persons were mostly professional blood donors, but included 40 persons whose blood specimens were received by air-express from Germany. The blood specimens of Negroids were kindly supplied by Dr. L. J. Unger, while the blood specimens of Chinese origin were obtained from New York Chinese by Dr. H. Chin.

RESULTS

In Table 1 are presented the reactions of anti-rh^w serum with blood specimens from 259 Caucasoid, 104 Negroid, and 31 Chinese persons classified according to their Rh-Hr blood types. Among the 259 Caucasians, there were 10, or 4.0 per cent, whose bloods were clumped by the anti-rh^w serum, while none of the

bloods from Negroes or Chinese were clumped by the serum. Moreover, all 10 blood specimens clumped by the anti-rh^w serum possessed the factor rh′, in conformity with the original report of Callender and Race.

The findings described strongly indicate that the factor rh^w is a partial antigen which is related to the factor Rh_1 in a manner similar to the relationship of partial antigen A_1 to the agglutinogen A. The parallel between the reactions of anti-rh^w and anti-A_1 serum is shown in Table 2.

The interpretation of Callender and Race of the reactions of anti-rh^w serum is given in their article as follows: "During the investigation of the Willis antibody (anti-C^w) it has become clear that about half the sera of the type called anti-C (anti-rh′) are really mixtures of anti-C and anti-C^w, the other half are

TABLE 2

ANALOGY BETWEEN THE REACTIONS OF SERUMS ANTI-rh^w AND ANTI-A_1

BLOOD OF TYPE	REACTION WITH SERUM		BLOOD OF SUBGROUP	REACTION WITH SERUM	
	Anti-rh′	Anti rh^w		Anti-A	Anti-A_1
Rh_1^w	+	+	A_1	+	+
Rh_1	+	−	A_2	+	−

TABLE 3

RACE'S INTERPRETATION OF THE REACTIONS OF ANTI-rh′ SERUMS

BRITISH NOMENCLATURE				INTERNATIONAL NOMENCLATURE			
Blood of Type	Reactions with Anti-rh′ Serums			Blood of Type	Reactions with Anti-rh′ Serum		
	Anti-C + Anti-C^w	Anti-C	Anti-C^w		Anti-rh′ + Anti rh^w	Anti-rh′	Anti-rh^w
C^w	+	−	+	Rh_1^w	+	−	+
C	+	+	−	Rh_1	+	+	−

pure anti-C. This finding provides the explanation of occasional discrepancies of behavior between different anti-rh′ sera which we have noted in the last two years. The two anti-C sera in use for testing of blood in this investigation are known to be of the mixed type, anti-C and anti-C^w. Consequently, cells positive with anti-Willis (anti-C^w) had to be positive with anti-C in use and, therefore, appear to have the antigen C. As a result of this, anti-C^w appears to be splitting off something from C, whereas in reality it was recognizing an alternate to C."

If we interpret these statements correctly, Callender and Race believe that the difference between antigens C and C^w is due to related chemical structures determined by corresponding genes of an allelic series, and that the cross reactions of various anti-rh′ serums with the two types of blood are due to the presence in these serums of *more than one type of antibody*. This is shown in Table 3, in terms of both the International and British nomenclatures. According to their concept, the ability of most anti-rh′ serums to clump blood of type Rh_1^w blood as

well as the ordinary type Rh_1 blood is explained on the same basis as the ability of group O serum (containing anti-A and anti-B) to clump group A as well as group B blood, namely, by the presence of *two* antibodies in the cross-reacting serums.

Attempts by Race, Sanger and Lawler[10] to verify their explanation of the cross-reactions of anti-rh′ serums with bloods of types Rh_1 and Rh_1^w, namely, that cross-reacting serums contain two antibodies have led to a number of paradoxes. (1) When these workers pooled anti-rh′ serum of the type that does not clump Rh_1^w blood with anti-rh^w serum, they found that the resulting polyvalent anti-rh′ + anti-rh^w serum could be fractionated into its two components by selective absorption with Rh_1^w and Rh_1 cells, respectively, as expected. On the other hand, ordinary anti-rh′ serum which clumps both Rh_1^w and Rh_1 bloods, and which according to Race *et al.*, should contain two antibodies, anti-rh′ and anti-rh^w, were completely absorbed by either Rh_1 or Rh_1^w cells, so that the two antibodies supposedly present could not be separated. They then suggested the highly improbable explanation that the postulated two antibodies in the latter serums are both present on the same molecule, implying that each antibody molecule has two different specific combining groups, one for rh′ and one for rh^w. (2) Since ordinary type Rh_1 blood lacks the rh^w component, it became difficult to explain the supposed presence of anti-rh^w in ordinary anti-rh′ serums. Race *et al.* consider that rh′ or C is a single antigen, and that it evokes not only its specific anti-C body but also, in addition, anti-C^w as an extra or "nonspecific" response. In support of this interpretation, they misquote the work of Landsteiner and van der Scheer[7] on the cross-reactions of antiserums against conjugated antigens containing pure meta-amino benzene sulphonic acid. Such antiserums cross-react with antigens containing ortho-amino benzene sulphonic acid, meta-amino benzene arsenic acid and meta-amino benzoic acid. However, Landsteiner,[6] like Ehrlich, conceived of antibodies as a mold which fits the antigen in a manner similar to the way a lock fits its key, or a plaster cast fits its corresponding mold. Obviously, a cast cannot have an impression which does not correspond to a structure on the mold, and similarly an antigen cannot stimulate an antibody for a structure which it does not contain itself. As Landsteiner[6] pointed out (on page 54 of his book), "The activity of antigens extends to structures which are chemically similar to those of the homologous antigen." This concept may be compared to the ability of a key made for one lock to open another lock of similar structure. The absorption experiments of Race *et al.* really show that the action of common anti-rh′ serums in giving cross-reactions with bloods of type Rh_1 and Rh_1^w is due to the presence of a *single kind of antibody molecule.* Under Landsteiner's concept, it is not necessary to assume that the antibody molecules each contain two different combining groups or that an antigen can stimulate an antibody to a structure it does not possess, but merely that Rh_1 and Rh_1^w bloods possess molecular structures of sufficient chemical similarity so that certain anti-serums for Rh_1 can produce a cross-reaction with Rh_1^w (Fig. 1). This concept receives support from observations that all of the heterozygous Rh_1^w bloods in our series were clumped less in-

tensely by our anti-rh′ serums than ordinary Rh_1 bloods. If, as Callender and Race state, there also exist serums which clump ordinary Rh_1 bloods but not Rh_1^w bloods, then it is apparent that ordinary Rh_1 bloods have a special property distinguishing them from Rh_1^w blood. If these special serums are designated as anti-rh^x, then ordinary Rh_1 blood would have to be designated as Rh_1^x as shown in Table 4.

Moreover, as shown in Table 4, the factors rh^w and rh^x are partial antigens within the factor rh′, just as rh′ and rh″ are partial antigens within the Rh_0-

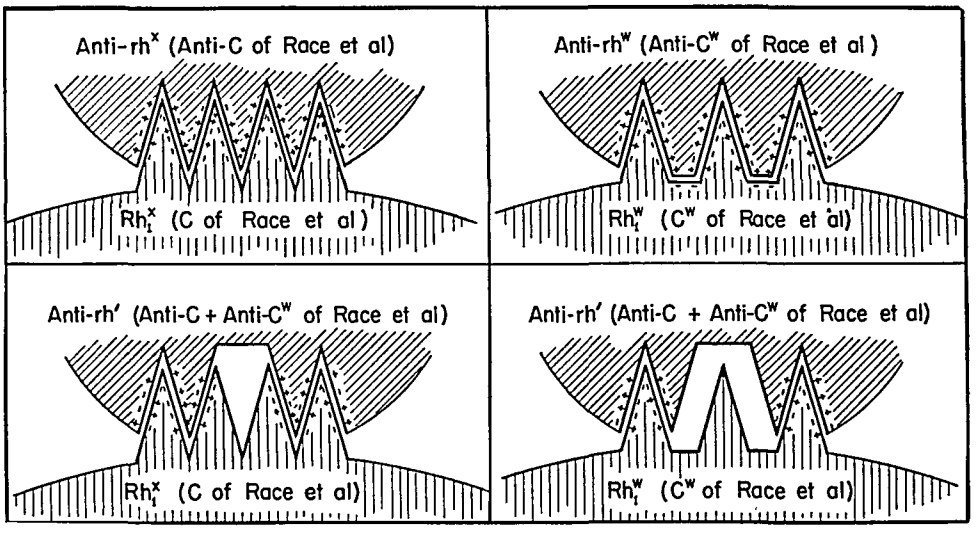


FIG. 1. Diagrammatic explanation of the present authors' concept of the reactions of anti-rh′ serums. When ordinary type Rh_1 blood (Rh_1^x, or C of Race *et al.*) is injected into a person having type rh blood, the antibody-producing cells first release a highly specific antibody such as anti-rh^x (or anti-C of Race *et al.*). Upon continued sensitization, the antibody-producing cells presumably become fatigued and produce a less perfect counterpart of the antigen such as anti-rh′ (incorrectly named anti-C + C^w by Race), which cross-reacts with Rh_1^w. Thus, the cross-reactions of anti-rh′ serum, according to our concept, is not due to the presence of two antibodies in the antiserum as postulated by Race, but to the presence of a single species of antibody capable of reacting with almost equal avidity with both agglutinogens. Obviously, if an ordinary type Rh_1 (or Rh_1^x) individual is sensitized with type Rh_1^w blood, the only type of antibody he can produce is some form of "pure" anti-rh^w, incapable of reacting with his own type Rh_1^x cells.

positive factors Rh_1 and Rh_2. It is inconsistent, therefore, on the part of Race *et al.* to explain rh^w on the basis of multiple alleles and rh′ and rh″ on the basis of linked genes. Incidentally, Landsteiner has emphasized repeatedly that there is not a one-to-one correspondence between the number of antibodies formed by the immunized animal and the number of specific structures within the antigen molecule, because, as pointed out above, the antibody behaves like a mold of the antigen.

Racial Distribution of the rh^w Factor

In Table 5 are summarized the available data on the rh^w factor in different populations, including the present study. Though the number of tests made to

date on persons of Negroid or Mongoloid derivation is regrettably small, the findings suggest that the rh^w factor is restricted to Caucasoid peoples, among whom its incidence ranges from approximately 2.5 to 8 per cent. The Basques appear to constitute an exception, since not a single rh^w blood was found among 167 Basques examined. If the latter results are confirmed in a larger series, it may be necessary to search for the origin of the rh^w factor in the Middle East.

TABLE 4

PRESENT AUTHORS' INTERPRETATION OF THE REACTIONS OF ANTI-rh′ SERUMS SHOWING THE ANALOGY BETWEEN SUBTYPES OF TYPE Rh_1 AND CERTAIN SUBDIVISIONS OF THE Rh_0-POSITIVE TYPE

BLOOD OF SUBTYPE*	REACTION WITH			BLOOD OF TYPE	REACTION WITH		
	Anti-rh′	Anti-rh^w	Anti-rh^x		Anti-Rh_0	Anti-rh′	Anti-rh″
Rh_1^w	+	+	−	Rh_1	+	+	−
Rh_1^x	+	−	+	Rh_2	+	−	+

* Type Rh_1^x blood is ordinary type Rh_1 blood.

TABLE 5

RELATIVE INCIDENCE OF FACTOR rh^w IN DIFFERENT RACES

POPULATION STUDIED	INVESTIGATORS	NUMBER OF PERSONS TESTED	REACTIONS WITH ANTI-rh^w	
			Positive (Per cent)	Negative (Per cent)
Caucasoids (New York City)	Wiener and Gordon (Present study)	259	4.0	96.0
Negroids (New York City)		104	0	100
Chinese (New York City)		31	0	100
Caucasians				
England	Race *et al.*[9]	1073	2.5	97.5
Czechoslovakia	Raska *et al.*[12]	181	7.7	92.3
Norway	Hartman *et al.*[5]	226	4.0	96.0
Holland	Lawler *et al.*[8]	205	2.4	97.6
Latvia	Race *et al.*[11]	172	3.5	96.5
Basques	Chalmer *et al.*[4]	167	0	100

Distribution of the Rh Genes Among Caucasoids in New York City

Among 1071 professional blood donors tested with antiserums for Rh_0, rh′, rh″, and hr′, the following distribution of the Rh-Hr phenotypes was found.

Type	*Number*	*Per Cent*
rh	154	14.4
Rh_1Rh_1	209	19.5
Rh_1rh	337	31.5
Rh_2	175	16.3
Rh_1Rh_2	154	14.4
Rh_0	27	2.5
rh′	11	1.0
rh″	4	0.4
Totals	1071	100

From this distribution of the Rh-Hr types the frequencies of the Rh genes can be calculated* as follows:[17]

$$r = \sqrt{\text{rh}} = \sqrt{0.1438} = 37.92 \text{ per cent}$$

$$r' = \sqrt{\text{rh}' + \text{rh}} - \sqrt{\text{rh}} = \sqrt{0.1541} - \sqrt{0.1438} = 1.34 \text{ per cent}$$

$$r'' = \sqrt{\text{rh}'' + \text{rh}} - \sqrt{\text{rh}} = \sqrt{0.1475} - \sqrt{0.1438} = 0.49 \text{ per cent}$$

$$R^0 = \sqrt{\text{Rh}_0 + \text{rh}} - \sqrt{\text{rh}} = \sqrt{0.1690} - \sqrt{0.1438} = 3.19 \text{ per cent}$$

$$R^1 = \sqrt{\text{Rh}_1 + \text{rh}' + \text{Rh}_0 + \text{rh}} - \sqrt{\text{Rh}_0 + \text{rh}} - \sqrt{\text{rh}' + \text{rh}} + \sqrt{\text{rh}}$$
$$= \sqrt{0.6891} - \sqrt{0.1690} - \sqrt{0.1541} + \sqrt{0.1438} = 40.24 \text{ per cent}$$

$$R^2 = \sqrt{\text{Rh}_2 + \text{rh}'' + \text{Rh}_0 + \text{rh}} - \sqrt{\text{Rh}_0 + \text{rh}} - \sqrt{\text{rh}'' + \text{rh}} + \sqrt{\text{rh}}$$
$$= \sqrt{0.3361} - \sqrt{0.1690} - \sqrt{0.1475} + \sqrt{0.1438} = 16.37 \text{ per cent}$$

Thus, $r + r' + r'' + R^0 + R^1 + R^2 = 99.55$ per cent, which is a remarkably close fit to the theoretical expectation, especially when one allows for the rare genes R^z and r^y.

Further evidence testing the theory of multiple alleles is obtained by calculating the expected frequencies of phenotypes Rh_1Rh_1, Rh_1rh and Rh_1Rh_2 from the gene frequencies, and comparing them with the observed frequencies.

$\text{Rh}_1\text{Rh}_1 = (R^1 + r')^2 - (r')^2 = (0.4158)^2 - (.0134)^2 = 17.3$ per cent

$\text{Rh}_1\text{rh} = 2(R^1 + r')(R^0 + r) - 2(r)(r') = 2(0.4158)(0.4111) - 2(.0134)(0.3792)$
$= 33.2$ per cent

$\text{Rh}_1\text{Rh}_2 = 2(R^1 + r')(R^2 + r'') - 2(r')(r'') = 2(0.4158)(0.1686) - 2(.0134)$
$(.0049) = 14.0$ per cent

It will be seen that the observed frequencies agree satisfactorily with the theoretically expected values.

As has already been pointed out, in tests to date, the factor rh^w occurred exclusively in combination with Rh_1, to form the complex agglutinogen Rh_1^w. Thus, the gene R^1 actually consists of two main variants R^{1w} and R^{1x}, just as the gene I^A determining agglutinogen A consists of two main variants I^{A_1} and I^{A_2}. The frequency of gene R^{1w} is readily calculated as follows:

$$R^{1w} = 1 - \sqrt{\text{rh}^w\text{-negative}}$$

For our New York City Caucasoids, therefore, $R^{1w} = 1 - \sqrt{0.9598}$

$$= 2.03 \text{ per cent}$$

$$\text{and } R^{1x} = R^1 - R^{1w} = 38.21 \text{ per cent}$$

COMMENT

At first, Callender and Race's misinterpretation of the cross-reactions of ordinary anti-rh′ serums with blood type Rh_1 blood as well as ordinary type Rh_1 blood misled us[19] into believing that the newly found factor corresponded

* It is important to note that the gene frequencies can be calculated without the aid of tests with anti-Hr serums.

to the hypothetical factor rh‴ previously discussed by one of us.[14] From our own study of the rh^w factor, however, it has become clear that rh^w actually identifies one of the numerous so-called "intermediate" Rh factors. Following the discovery of the three Rh factors, Rh_0, rh′ and rh″, and the 8 Rh types determined by them,[13, 16] it was found that occasional specimens of blood gave weak reactions with one or more of the reagents, anti-Rh_0, anti-rh′, and anti-rh″, indicating the existence of variants of each of the Rh factors.[14, 15] As shown in this paper, rh^w accounts for a certain percentage of the variants of the rh′ factor. That more variants of rh′ exist follows from our observation that rh^w is apparently absent in Negroids despite the higher frequency among such persons of bloods giving weak reactions with anti-rh′ serums.[20] Race *et al.*[10] have in fact recently identified several additional variants of the blood type Rh_1, in addition to Rh_1^w.

No doubt a similar situation exists regarding variants of factors Rh_0 and rh″, and probably also hr′ and hr″. It must be emphasized that a specific name for an "intermediate" Rh type is not justified except when a specific antiserum has been found, as in the case of type Rh_1^w blood containing the specific factor rh^w. In the present state of our knowledge concerning the Rh_0 variants, the name D^u is misleading and possibly incorrect, as long as no specific factor or factors corresponding to D^u have been isolated.

SUMMARY

Ten of 259 bloods (4.0 per cent) of Caucasoid origin were clumped by anti-rh^w serum while not one specimen of blood of 104 Negroid and 31 Chinese persons was clumped by anti-rh^w serum. All 10 bloods clumped by rh^w antiserums contained the factor rh′, indicating that rh^w is a partial antigen associated with factor rh′, especially in bloods containing agglutinogen Rh_1, just as factors rh′ and rh″ are partial antigens associated with Rh_0 in particular Rh_0-positive bloods. These results confirm previous observations indicating the existence of further variants of the Rh factor in addition to the eight standard varieties, presumably transmitted by corresponding allelic genes.

REFERENCES

1. Broman, B.: Personal communication to the senior author.
2. Callender, S. T., and Race, R. R.: A serological and genetical study of multiple antibodies formed in response to blood transfusion by a patient with lupus erythematosus diffusus. Ann. Eugenics, **13:** 102–117, 1946.
3. Callender, S. T., Race, R. R., and Paykoc, Z. V.: Hypersensitivity to transfused blood. Brit. M. J., **2:** 83–84, 1945.
4. Chalmer, J. M., Ikin, E. W., and Mourant, A. E.: Basque blood groups. Nature, **162:** 27, 1948.
5. Hartman, O., Mourant, A. E., and Race, R. R.: The Rh genotypes in a series of Oslo blood donors. Acta path., **24:** 330–333, 1947.
6. Landsteiner, K.: The Specificity of Serological Reactions. Cambridge, Mass.: Harvard University Press, 310 pp., 1945.
7. Landsteiner, K., and van der Scheer, J.: On cross reaction of immune sera to azoproteins. J. Exper. Med., **63:** 325–339, 1936.
8. Lawler, S. D., and van Loghem, J. J., Jr.: The rhesus antigen C^w, causing hemolytic disease of the newborn. Lancet, **2:** 545, 1947.
9. Race, R. R., Mourant, A. E., Lawler, S. D., and Sanger, R.: The Rh chromosome frequencies in England. Blood, **3:** 679–695, 1948.

10. RACE, R. R., SANGER, R., AND LAWLER, S. D.: Allelomorphs of the Rh gene C. Heredity, **2:** 237–240, 1948.
11. RACE, R. R., SANGER, R., LAWLER, S. D., AND KEETCH, D. V.: Blood groups of Latvians; A_1-A_2-B-O, M-N and Rh. Ann. Eugenics, **14:** 134–138, 1948.
12. RASKA, K., SANGER, R., LAWLER, S. D., AND RACE, R. R.: Krevní skupiny a Rh genotypy českém obyvatelstva (Blood groups and Rh genotypes in the Czech population). Čas. lék. česk., **86:** 1517, 1947.
13. WIENER, A. S.: Genetic theory of the Rh blood types. Proc. Soc. Exper. Biol. and Med., **54:** 316–319, 1943.
14. WIENER, A. S.: The Rh series of allelic genes. Science, **100:** 595–597, 1944.
15. WIENER, A. S., DAVIDSOHN, I., AND POTTER, E. L.: Heredity of the Rh blood types; observations on the relation of factor Hr to the Rh blood types. J. Exper. Med., **81:** 63–72, 1945.
16. WIENER, A. S., AND LANDSTEINER, R.: Heredity of variants of the Rh type. Proc. Soc. Exp. Biol. and Med., **53:** 167–170, 1943.
17. WIENER, A. S., SONN, E. B., AND BELKIN, R. B.: Heredity of the Rh blood types. J. Exper. Med., **79:** 235–253, 1944.
18. WIENER, A. S., AND SONN-GORDON, E. B.: Réaction transfusionnelle hémolytique intra-groupe due a un hémagglutinogène jusqu'ici non décrit. Rev. d'Hémat., **2:** 3–10, 1947.
19. WIENER, A. S., SONN-GORDON, E. B., AND HANDMAN, L.: Heredity of the Rh blood types; additional family studies with special reference to the theory of multiple allelic genes. J. Immunol., **57:** 203–210, 1947.
20. WIENER, A. S., UNGER, L. J., AND SONN, E. B.: New data on the distribution of the Rh blood types. Proc. Soc. Exper. Biol. and Med., **58:** 89–92, 1945.

ACUTE HEMOLYTIC ANEMIA ASSOCIATED WITH ELEVATED TITER OF COLD AGGLUTININS

Report of Case*

IRA L. RUBIN, M.D., ABRAHAM S. JACOBSON, M.D., AND LEO M. MEYER, M.D.

From the Medical Service, Bronx Veterans Administration Hospital, New York, New York

Increased cold agglutinin titers were first noted in the presence of a diffuse bronchopneumonia in 1918 by Clough and Richter.[1] It was not until 1943, however, that the association of elevated cold agglutinin titers with primary atypical pneumonia was made by Peterson, Ham and Finland.[11] Since then, many reports have appeared confirming this finding.[5, 7, 9, 10, 12]

The hemolytic properties of the cold agglutinin were first observed by Salen[13] and later confirmed by Stats and his co-workers.[14–16] Coincident with these observations, reports of the occurrence of hemolytic phenomena in patients with atypical pneumonia and elevated cold agglutinin titers began to appear.[3, 4, 8] In these cases, the patients had received sulfonamide therapy, and since sulfonamides alone can induce hemolytic reactions, the etiologic role of the cold agglutinin was inconclusive. Ginsberg[6] reported the first case of acute hemolytic anemia during the course of primary atypical pneumonia in which no sulfonamide was given. Colmers and Snavely[2] reported a similar case and concluded that their case together with Ginsberg's demonstrated that "the acute hemolytic anemia of primary atypical pneumonia is a distinct entity due directly to the action of cold agglutinins on the red cells of the patient."

We present the clinical record of a patient with severe hemolytic anemia during the course of primary atypical pneumonia. No sulfonamide therapy was given to him.

REPORT OF CASE

A 25 year old white male was admitted to the hospital April 18, 1947 with the chief complaint of marked weakness and fatigability of two weeks' duration. In September 1944, while serving in the Navy, he developed thrombophlebitis of the left leg. He was kept at bed rest for three weeks and was then transferred to the Medical Service for a short period because of "anemia". After return to duty, he was well until January 1946, when he had a recurrence of thrombophlebitis in his left leg and was hospitalized for a nine month period. He was given a medical discharge from the Navy because of the thrombophlebitis and went to work as an explosive handler at an arsenal. Three weeks prior to admission, he had an infection of the upper respiratory tract and felt weak. A few days later he consulted a physician because of an infected sebaceous cyst of his neck which was incised and drained. One week prior to admission the physician noted pallor and weakness. The patient noticed that his urine was darker than usual. A blood count on April 16, 1947 was reported as showing 1.95 million red blood cells, 33 per cent hemoglobin, color index 0.82

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

Received for publication, March 14, 1949.

and 43,800 white blood cells per cu.mm. with 8 per cent myeloblasts, 88 per cent leukocytes and 4 per cent lymphocytes, suggesting chronic myelogenous leukemia. At no time during his present illness did the patient receive any sulfonamide medication.

On physical examination, the patient appeared acutely ill. The conjunctivae were markedly pale. The lungs were clear to percussion and auscultation and a blowing systolic murmur was heard at the basal area. The tip of the spleen was palpable in the right lateral position. No residua were noted of the previous thrombophlebitis.

The red blood count on admission was 960,000 per cu. mm., hemoglobin 3.2 Gm.; white blood count 20,000 per cu. mm. with 97 per cent neutrophils including 4 per cent myelocytes, 7 per cent metamyelocytes, and 3 per cent lymphocytes. Urinalysis was normal. Urinary urobilinogen was present in a dilution of 1 to 10 on four occasions between April 24 and September 23, 1947. The icterus index was 12.3 with an indirect van den Bergh of 1.8 mg. and a negative direct van den Bergh.

Because of the patient's marked anemia and poor general condition, blood was drawn for typing and cross-matching in preparation for transfusion. Auto-agglutination was noted at room temperature in the syringe. With the aid of a warm syringe, successful typing and cross-matching was performed. The patient was transfused with 1000 ml. of whole blood, type A, Rh-positive, without reaction. The patient's cold agglutinin titer was then determined (April 18, 1947) in view of the presence of agglutination at room temperature. It was found to be positive in a dilution of 1:1024 at 5 C.

The patient was febrile during the first week of hospitalization with the temperature reaching 100 F. daily. He was given penicillin intramuscularly during this first week. An x-ray film of the chest on April 22, four days after admission, showed an irregular zone of increased density in the lower segment of the right lung field. Serial x-ray films of the chest revealed gradual clearing of this infiltrate, but it was not until the second week of July that the lungs were cleared completely. At no time during the patient's stay in the hospital were there any abnormal findings on physical examination of the chest or any symptoms referable to the respiratory tract.

The red blood cell count and hemoglobin level rose rapidly after the initial transfusion, and no further transfusions were necessary. His white blood cell and differential counts quickly returned to normal values. Sternal marrow aspirations (April 20, 1947) confirmed the incorrectness of the admission diagnosis of leukemia and revealed a marked erythropoietic reaction with signs of regeneration and immaturity of the nucleated red cells and a mild myeloid reaction. The cold agglutinin titer fell to 1:32 in one week's time (April 24). The Donath-Landsteiner test was normal. On May 9, the cold agglutinin titer was 1:4. The spleen gradually increased in size until its edge could be felt 2 cm. below the costal margin.

The patient was discharged on June 11, 1947 and was seen several times thereafter. On each occasion, he had a mild anemia (erythrocytes 3,010,000 to 3,900,000; hemoglobin 10.2 to 12.8 Gm.), slight reticulocytosis, (1.8 to 2.4 per cent), elevated icterus index (13 to 26) and a palpable spleen. On July 14, no hemoglobinuria was found after he had exercised moderately for one and one-half hours. When examined Dec. 17, 1947, he was pale, the conjunctivae were jaundiced, the spleen was still palpable, anemia was present and the icterus index was 26.

Inquiry at the arsenal where he had been employed revealed no exposure to any known toxic agent.

DISCUSSION

Most patients with atypical pneumonia are acutely ill with respiratory symptoms at the time of the development of a hemolytic reaction and the association readily becomes apparent to the physician. However, in the case described, the patient had no respiratory symptoms at the time of his admission to the hos-

pital or during his hospitalization. Physical examination of the lungs was normal even after the pneumonitis had been found by x-ray examination.

We believe that our patient has an acquired hemolytic anemia as indicated by the persistently palpable spleen, elevated reticulocyte count, elevated icterus index and the failure of the red blood count to rise above 4 million per cu. mm. It seems reasonable that such a patient would be more likely to develop an acute hemolytic episode in the presence of cold agglutinins in the blood.

It should be noted, however, that repeated erythrocyte fragility tests during the patient's hospital stay were normal and that the Coombs tests performed after the patient's discharge from the hospital were negative.

The possibility of the presence of a high cold agglutinin titer should be investigated in any patient with an acute hemolytic anemia of unknown etiology in spite of the absence of signs and symptoms of pulmonary involvement. This is especially important in view of the possibly dangerous effects of chilling in these patients.[2]

SUMMARY

The case history is presented of a patient with primary atypical pneumonia whose only complaints and signs were referable to a severe hemolytic process. The presence of an atypical pneumonia was not discerned until the examination of a routine roentgenogram of the chest. Tests for cold agglutinins were performed after auto-agglutination was noted while cross-matching and typing for transfusion upon admission to the hospital. This incidental discovery of a cold agglutinin titer, in a dilution of 1:1024, led to a proper evaluation of the nature of the hemolytic crisis. This patient had not received sulfonamide medication during the course of his illness. In addition, it is believed that the patient has an acquired hemolytic anemia.

REFERENCES

1. Clough, M. C., and Richter, I. M.: Study of autoagglutinins occurring in human serum. Bull. Johns Hopkins Hosp., **29:** 86–93, 1918.
2. Colmers, R. A., and Snavely, J. G.: Acute hemolytic anemia in primary atypical pneumonia produced by exposure and chilling. New England J. Med., **237:** 505–511, 1947.
3. Dameshek, W.: Cold hemagglutinins in acute hemolytic reactions in association with sulfonamide medication and infection. J.A.M.A., **123:** 77–80, 1943.
4. Finland, M., Peterson, O. L., Allen, H. E., Samper, B. A., and Barnes, M. W.: Cold agglutinins; cold isohemagglutinins in primary atypical pneumonia of unknown etiology with note on occurrence of hemolytic anemia in these cases. J. Clin. Investigation, **24:** 458–473, 1945.
5. Florman, A. L., and Weiss, A. B.: Serologic reactions in primary atypical pneumonia. J. Lab. and Clin. Med., **30:** 902–910, 1945.
6. Ginsberg, H. S.: Acute hemolytic anemia in primary atypical pneumonia associated with high titer cold agglutinins. New England J. Med., **234:** 826–829, 1946.
7. Heintzelman, J. H. L., and Seligmann, A. W., Jr.: Evaluation of cold agglutination test in primary atypical pneumonia. U. S. Nav. M. Bull., **43:** 433–437, 1944.
8. Horstmann, D. M., and Tatlock, H.: Cold agglutinins; a diagnostic aid in certain types of primary atypical pneumonia. J. A. M. A., **122:** 369–370, 1943.
9. Humphrey, A. A.: Cold hemagglutination test in diagnosis of primary atypical pneumonia. U. S. Nav. M. Bull., **43:** 1117–1127, 1944.
10. McNeil, C.: The relationship of cold agglutinins to the course of primary atypical pneumonia. Am. J. M. Sc., **209:** 48–54, 1945.

11. PETERSON, O. L., HAM, T. H., AND FINLAND, M.: Cold agglutinins (autohemagglutinins) in primary atypical pneumonias. Science, **97:** 167, 1943.
12. RICH, C. B., RAE, M. V., AND MCGOEY, C. J.: Cold agglutinins and the pneumonias. Canad. M. A. J., **51:** 239–240, 1944.
13. SALÉN, E. B.: Thermostabiles, nicht komplexes (Auto-) Hämolysin, bei transitorischer Kältehämoglobinurie. Acta med. Scandinav., **86:** 570–592, 1935.
14. STATS, D.: Cold agglutinated erythrocytes; hemolytic effect of shaking. Proc. Soc. Exper. Biol. and Med., **54:** 305–306, 1943.
15. STATS, D.: Cold hemagglutination and cold hemolysis; hemolysis produced by shaking cold agglutinated erythrocytes. J. Clin. Investigation, **24:** 32–42, 1945.
16. STATS, D., AND BULLOWA, J. G. M.: Cold hemagglutination with symmetric gangrene of tips of extremities; report of a case. Arch. Int. Med., **72:** 506–517, 1943.

MASSIVE STILBAMIDINE THERAPY OF MULTIPLE MYELOMA

Report of Case*

THOMAS A. HAEDICKE, MAJOR, M.C., and EZRA M. GREENSPAN, M.D.

From the Medical Service, Walter Reed General Hospital, Washington, D. C.

Stilbamidine was first used by Snapper[6] in the treatment of multiple myeloma because of the characteristic hyperglobulinemia which is also present in kala-azar, a disease that had responded favorably to the same drug. In patients with severe bone pain associated with generalized myeloma, he noted a "specific influence" of stilbamidine on symptoms, provided that a low-protein dietary regime was maintained. Snapper[6, 7] and Snapper and Schneid[9] observed the presence of large basophilic inclusion granules in the cytoplasm of many of the myeloma cells in the bone marrow and, in a few instances, in the peripheral blood. These usually appeared from 3 to 14 weeks after treatment was begun in myeloma patients having a hyperglobulinemia. With the aid of special staining technics, ultra-violet microscopy, and solutions of the enzyme ribonuclease, Snapper and associates[8] demonstrated that the basophilic granules were probably precipitates composed of stilbamidine and ribose nucleic acid.

The present case of multiple myeloma is being reported because of the patient's tolerance to an unusually large amount of stilbamidine administered by intravenous drip, temporary symptomatic improvement, and transient changes in the cytoplasm of the plasma cells. Because of the tendency of the drug to affect the nerves, especially the trigeminal, and because of potential renal toxicity, Snapper[10] advised a total dose of 2.5 Gm. over a three week period, less than one fourth of the total dose of stilbamidine given to our patient.

REPORT OF CASE

The patient, a 34 year old Italian prisoner of war, noticed a sudden severe low back pain in August 1945, after picking up a light weight. The pain was lancinating, radiated to both hips, and was aggravated by coughing, sneezing and excessive motion. He was incapacitated and lost approximately 20 pounds in weight during the following month. The pain became more severe and occurred in all four extremities and in the chest during the following month.

Laboratory data. October 10, 1945; red blood cells, 3,400,000 per cu. mm., hemoglobin 9.6 Gm. (Turner's method); leukocyte count 9200; neutrophils, 62 per cent; lymphocytes, 36 per cent; monocytes, 2 per cent; sedimentation rate, 42 mm. per hour (Wintrobe); serum albumin, 1.6 Gm. and globulin, 6.8 Gm.; blood calcium, 11.8 mg. and phosphorus, 3.4 mg. per 100 ml.; acid phosphatase, 1.4 Bodansky units; urinalysis, one to two plus albuminuria, no Bence-Jones protein; gastric analysis, no free hydrochloric acid, even after injection of histamine. X-ray examination revealed "collapse of the body of the twelfth thoracic vertebra and widespread circular osteolytic lesions of the skull, spine, ribs, and pelvis." Intravenous pyelogram and x-ray films of the chest were negative. December 1945: X-ray films revealed extensive destructive changes in the lower cervical and thoracic vertebrae

*Published under the auspices of the Surgeon General, U. S. Army, who does not necessarily assume responsibility for the professional opinions expressed by the authors.

Received for publication, February 14, 1949.

with collapse of five thoracic vertebrae, severe destructive changes of almost all the ribs, both scapulae, both clavicles, the upper end of the right humerus and all bones of the pelvis. January 1946: sedimentation rate, 59 mm. per hour (Wintrobe); total proteins, 10 Gm.; albumin, 2.4 Gm., and globulin, 7.6 Gm. per 100 ml.; alkaline phosphatase, 19 Bodansky units; albuminuria, four plus.

The patient was admitted to Walter Reed General Hospital on January 21, 1946 complaining of generalized bone pain, anorexia, loss of weight and weakness. He was bedridden and emaciated. February 1946, blood chemical values: urea nitrogen, 15 mg.; uric acid, 3.8 mg.; calcium, 10.5 mg.; phosphorus, 3.3 mg.; sedimentation rate, 30 mm. per hr. (Wintrobe); icteric index, 25; alkaline phosphatase, 1.6 Bodansky units; total proteins, 7.2 Gm.; albumin, 2.8 Gm.; globulin, 4.4 Gm. per 100 ml.; erythrocytes, 4,100,000 per cu. mm.; hemoglobin, 12 Gm.; leukocyte count, 6900; neutrophils, 59 per cent; lymphocytes, 40 per cent; eosinophils, 1 per cent; formol-gel test, positive in one minute. Urinalysis was negative for albumin; 24-hour calcium excretion on a ward diet was 1.25 Gm. Sternal marrow aspirated on February 22, 1946 was reported as normal. On March 13, 1946, a biopsy of sternal marrow revealed nests of "normal marrow cells of no diagnostic significance". A biopsy, however, of marrow from the left ninth rib revealed a marked increase of plasma cells, characteristic of multiple myeloma.

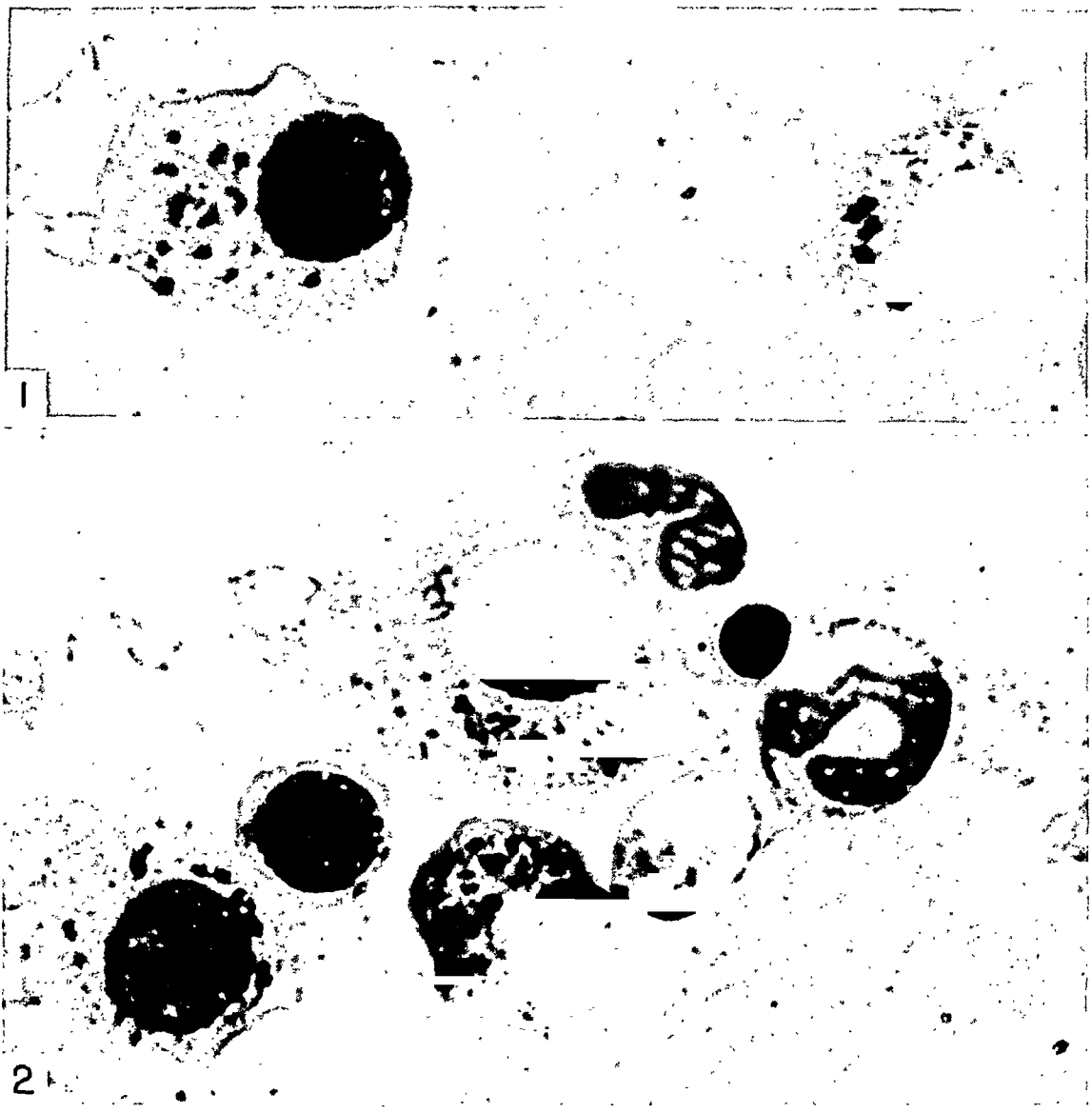
At the suggestion of Dr. Snapper, intravenous stilbamidine diisothionate therapy was begun. The initial dose of 40 mg. was given on April 30, 1946. This was gradually increased until a dose of 150 mg. was reached. A total of 1.365 Gm. was administered without significant symptoms over a three-week period. It was noted that after the fifth and sixth injections, the patient appeared strikingly improved. The generalized bone pain disappeared, but the low back pain persisted and he was able to move about in bed. Subsequent serum studies revealed little change in the blood proteins. June 6, 1946: total blood proteins, 9.6 Gm.; albumin-globulin ratio 1:4; calcium, 9.6 mg.; and phosphorus, 2.6 mg. per 100 ml.

A second course of daily stilbamidine diisothionate injections was begun on June 10, 1946. Each injection consisted of 250 ml. of glucose in distilled water, was given by intravenous drip and required 20 minutes for administration, the solution being protected from direct sunlight. The patient was given 150 mg. each day for 30 days. During the latter period of this course of injections, he was able to walk freely around the ward, and with the aid of a cane, on the hospital grounds. On July 16, 1946, he developed early signs of dissociated, bilateral anesthesia over the sensory distribution of the trigeminal nerve. There was loss of light touch, associated with numbness and tingling, but with preservation of normal pain and temperature sensation. This did not afford the patient any serious disability. It slowly abated during the ensuing nine months. On July 10, 1946, a sternal bone marrow aspiration revealed the presence of 15 per cent myeloma cells, approximately half of which showed peculiar basophilic inclusion granules in the cytoplasm (Figs. 1 and 2). During the middle of July 1946, another course of stilbamidine was begun, consisting of 150 mg. each day for a total of 30 days. The basophilic granules in the myeloma cells were observed to persist during August. Following the third course of stilbamidine therapy, the patient suffered a relapse of pain, which was principally confined to the lower lumbar area. In late August 1946, the patient developed paraplegia below the level of the twelfth thoracic vertebra with involvement of the bladder. He continued to lose weight and required intermittent transfusions for maintenance of an adequate red blood cell level. X-ray films of the bones revealed no tendency to recalcification.

A fourth course of daily infusions of stilbamidine diisothionate by intravenous drip was begun early in September 1946. The first two daily doses of 300 mg. were tolerated, without acute toxic reaction. The dose was then reduced to the usual 150 mg.; however, after six days, because of bronchopneumonia it was necessary to discontinue therapy. The pneumonia subsided after treatment with penicillin and transfusions.

On October 18, 1946, the patient was given the first of seven daily injections of 150 mg. of pentamidine hydrochloride (Merck) (total dose 1.05 Gm.). During treatment a marked

exacerbation of generalized tenderness occurred in all portions of the bones of the trunk and extremities. Four weeks after this treatment, examination of the sternal marrow did not reveal the presence of the basophilic granules seen previously in the plasma cells during stilbamidine therapy. The patient's condition continued unchanged until Jan-



FIGS. 1 AND 2. Basophilic inclusion granules in cytoplasm of myeloma cells after massive stilbamidine therapy. Wright's stain. × 1500.

uary, 1947, when he was again given a course of stilbamidine, 150 mg. daily for a total of 22 doses. On January 10, 1947, he sustained a spontaneous fracture in the upper third of the left femur without any significant preceding trauma. This healed slowly during the ensuing months. Two aspirations of the sternal marrow following cessation of stilbamidine therapy in February failed to reveal the basophilic granules originally seen in the cytoplasm of the plasma cells after the earlier courses of stilbamidine therapy.

In June 1947, the blood chemical values were: urea nitrogen, 19 mg.; creatinine, 1.8 mg.; blood sugar, 96 mg.; total proteins, 11.5 Gm.; albumin, 2.2 Gm.; globulin, 9.3 Gm.; albumin-globulin ratio, 1/4; prothrombin time, normal; alkaline phosphatase, 5.9 Bodansky units; cephalin flocculation, negative; thymol turbidity, negative; calcium, 12.5 mg.; red blood cells, 3,150,000 per cent; hemoglobin, 56 per cent; leukocytes, 6000 per cent; neutrophils, 63 per cent; lymphocytes, 30 per cent; monocytes, 2 per cent; eosinophils, 3 per cent; basophils, 2 per cent; chlorides, 510 (NaCl). Urinalysis revealed a trace of albumin, many red blood cells and white blood cells but no Bence-Jones protein. Progressive cachexia continued. Because of the paraplegia and cystopyelitis, further stilbamidine therapy was considered unjustified. From April 1946 to February 1947, he had received a total of 14.865 Gm. of stilbamidine diisothionate and 1.05 Gm. of pentamidine hydrochloride.

At the insistence of the patient and his family, he was flown to Rome, Italy, on July 3, 1947. He withstood the trip without event but expired three weeks later under unknown circumstances at a Red Cross Hospital.

COMMENT

During 16 months of observation we noted several interesting effects of stilbamidine treatment in multiple myeloma. As in Snapper's case[7] there was striking symptomatic improvement, our patient obtaining marked relief of generalized bone pain within four to six days of the onset of treatment. Although Geschickter[3] has described a well established cycle of pain in multiple myeloma, the remission of pain in our patient was believed to be the result of treatment. There was no significant relief in local pain from nerve root impingement, secondary to compression fractures of the lower vertebrae. Restriction of the patient's protein intake was not instituted despite Snapper's[6] previous suggestion that this was a requisite for obtaining symptomatic remission.

Stilbamidine diisothionate in doses of 150 and 300 mg. and pentamidine hydrochloride in doses of 150 mg. by intravenous drip were well tolerated without syncope, nausea, vomiting or other signs of immediate toxicity. Acute toxic manifestations have been reported[5] to occur frequently after single injections by syringe in these doses. No evidence of hepatic or renal toxicity was observed during the administration of 14.86 Gm. of stilbamidine in nine months in this patient. He developed the characteristic late (3 to 14 weeks after onset of treatment) dissociated neuropathy[4,7] over the face. Repeated treatment did not produce any aggravation of this peculiar lesion, which slowly diminished in intensity. These findings are considered of interest since our patient received approximately four times the recommended dosage. The apparent lack of toxic side actions may be explained by administration of the drug by infusion by intravenous drip, in contrast to the high blood levels known to be present during the first 15 minutes after a single injection of stilbamidine by syringe.[2]

Basophilic inclusions in the plasma cells were first observed in the bone marrow eight weeks after the onset of stilbamidine therapy and after a total of 5 Gm. had been administered. They persisted for two months, during which time the patient received the major portion of his therapy and attained maximum symptomatic improvement. No alterations of blood proteins were detected during this period, nor was any regression noted in the osteolytic lesions. Subsequent shorter courses of therapy and a course of pentamidine (1.05 Gm.) did not evoke granule formation.

In spite of symptomatic response and cytologic changes following treatment, and a survival of 23 months from the clinical onset of the disease, no conclusions may be drawn concerning the alteration of the clinical course in this case. The survival time was only four months longer than the average in a large series of myeloma patients recently reviewed.[1] Nevertheless, because stilbamidine may be of value in affording symptomatic relief in patients with multiple myeloma and intractable generalized bone pain, this drug deserves further trial.

SUMMARY

Massive stilbamidine therapy by intravenous infusion in a patient with diffuse multiple myeloma produced rapid symptomatic remission of generalized bone pain. A total of 14.86 Gm. was administered during a nine-month period without producing any evident visceral toxicity. Basophilic cytoplasmic inclusion bodies were produced in approximately fifty per cent of the myeloma cells during a two-month period. The patient survived for 23 months from the onset of symptoms and 15 months after treatment was begun.

REFERENCES

1. Bayrd, E. D., and Heck, F. J.: Multiple myeloma; review of 83 proved cases. J. A. M. A., **133**: 147–157, 1947.
2. Fulton, J. D., and Goodwin, T. W.: Studies on the estimation, absorption and precipitation of stilbamidine. J. Pharmacol. and Exper. Therap., **84**: 34–41, 1945.
3. Geschickter, Charles F., and Copeland, M.: Tumors of the bones. New York: American Journal of Cancer, pp. 456–457, 1936.
4. Napier, L. E., and Sen Gupta, P. C.: Peculiar neurological sequel to administration of 4:4′-diamidino-diphenyethylene. Indian M. Gaz., **77**: 71–74, 1942.
5. Napier, L. E., Sen Gupta, P. A., and Sen, G. N.: Treatment of kala-azar by diamidino stilbene; analysis of 101 cases. Indian M. Gaz., **77**: 321–338, 1942.
6. Snapper, I.: On the influence of stilbamidine on multiple myeloma. J. Mt. Sinai Hosp., **13**: 119–127, 1946.
7. Snapper, I.: Stilbamidine and pentamidine in multiple myeloma. J. A. M. A., **133**: 157–161, 1947.
8. Snapper, I., Mirsky, A. E., Ris, H., Schneid, B., and Rosenthal, M.: Development of inclusion bodies containing ribose nucleic acid in myeloma cells after injection of stilbamidine. Blood, **2**: 311–322, 1947.
9. Snapper, I., and Schneid, B.: On the influence of stilbamidine upon myeloma cells. Blood, **1**: 534–536, 1946.
10. Snapper, I., and Schneid, B.: On the treatment of multiple myeloma with 2-hydroxy stilbamidine. Blood, in press.

LEIOMYOMA ANGIOMATOSUM OF THE MESENTERY

REPORT OF CASE*

PH. H. HARTZ, M.D., AND FRANS R. VAN DE STADT, M.D.
From the Public Health Service, Curaçao, N. W. I.

Among tumors of the mesentery, if one excludes the lymphangiomas and lymphangiomatous cysts, there are decidedly few vascular neoplasms and neoplasms in which the vascular component plays an important part. Kaufmann[6] cites only three such cases reported before 1930. In Beitzke's[2] case of angiosarcoma, the new growth probably originated in the retroperitoneal tissue and invaded the mesentery and, therefore, cannot be considered as a primary mesenteric neoplasm.

The following case is reported not only because of the rarity of angiomatous mesenteric neoplasms, but also because of its peculiar histologic structure.

REPORT OF CASE

Clinical data. A 59 year old obese male Negro was admitted to the hospital with the following history. For one year he felt something like a ball in his abdomen. It could be moved from the left to the right side but usually was localized on the left side. The tumor was moderately painful and when situated medially it often caused urinary urgency. Micturition was frequent but defecation was normal. The left side of the abdomen contained a slightly tender freely movable tumor. Examinations of blood, urine and feces and roentgenograms of the kidneys were negative. On abdominal exploration, a somewhat nodular tumor was found in the mesentery about 15 cm. from the duodenojejunal flexure. The tumor was removed together with 25 cm. of jejunum. The postoperative course was uneventful and the patient was discharged after eight days. One year later he was in excellent health.

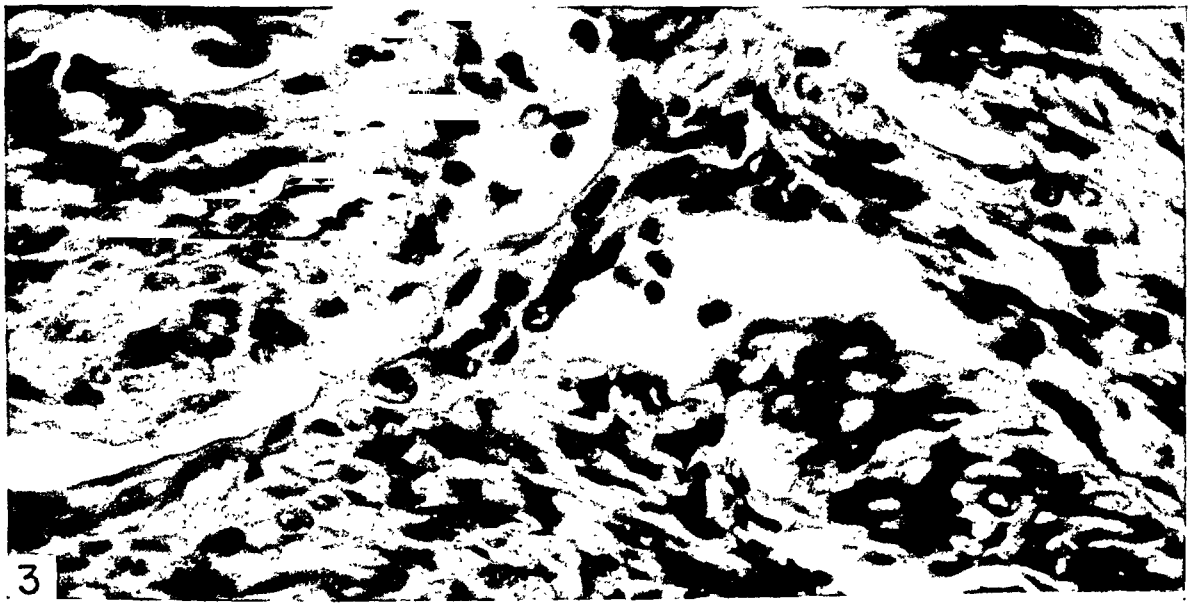
Gross appearance. The tumor measured 8 x 7.6 x 6 cm. and was covered by a thin capsule consisting of the more superficial layers of the mesentery. It contained many wide blood vessels and in several places a little adipose tissue. Except in a few places, the capsule could easily be stripped from the tumor. The intestine was freely movable except for a segment 4 cm. long which was firmly attached to the tumor. Here the growth had reached the mesenteric attachment of the intestine and was in direct contact with the thinned-out muscular coat of the jejunum. The surface of the tumor was smooth except for a few nodules and the consistency was firm. When the tumor was incised, much blood escaped. The cut surface showed pale, grayish brown to red bulging areas with distinct whorls, as in myoma, and darker brownish red areas some of which had a spongy appearance. No whorls were observed in the darker areas. On the cut surface of the formalin-fixed specimen the lighter colored areas contained a few small dark brown spots.

Microscopic appearance. Thin blocks, taken from different parts of the tumor, were fixed in Bouin's fluid, cleared in methyl benzoate-celloidin and embedded

* Received for publication, February 2, 1949.







FIGURES 1-3

in Tissuemat. Sections were stained with Masson's tetrachrome, using the modification of Larson and Levin; with the azan stain; with Lillie's modification of Gomori's method for the impregnation of reticulin and with Weigert's stain for elastic fibers.

The microscopic examination disclosed that the peculiar pattern observed grossly was caused by intermingling of two elements in various proportions: (1) bundles of nonstriated muscle fibers and (2) thin-walled, generally wide blood vessels which in many places strongly resembled cavernous tissue. The bundles in which the muscle fibers were arranged were of various thicknesses and ran in all directions. In the lighter colored areas that had a whorled appearance, the muscle fibers predominated, while in the darker areas the wide vessels were separated by thin trabeculae of muscular and connective tissue.

The muscle fibers generally contained the typical elongated nuclei with rounded ends and distinct nucleoli; their protoplasm contained conspicuous myofibrils. In many places large cells with large, atypical, often irregularly indented, segmented or vacuolated nuclei were observed. Although these large cells occurred also in the larger bundles, they were most often observed in the thinner trabeculae and in the places where the neoplastic tissue was edematous. In such places there were also large oval cells with one or two nuclei and without myofibrils. Mitotic divisions were very rare; only after examining many slides was a single mitotic figure found in a muscle fiber.

In the larger bundles of muscles there were only thin connective tissue fibers separating individual muscle cells. They stained well with aniline blue and were brought out distinctly by the Gomori-Lillie method. The arrangement of the reticular fibers was typical for nonstriated muscular tissue. In various places part of the muscle fibers was replaced by more or less hyaline connective tissue, and several thin trabeculae between blood vessels consisted of loose connective tissue, containing only a few muscle fibers. Elastic fibers were irregularly distributed throughout the neoplasm. Sometimes they were in direct contact with the vascular endothelium but the majority were found in the muscle bundles where they were arranged either parallel to the muscle cells or in small networks. The blood vessels consisted only of endothelium; their diameters varied within wide margins. No separate capillary basement membrane was found, the endothelial cells lying on the sheaths and networks of reticular fibers binding the smooth muscle cells. The endothelial cells were generally flattened and no mitotic divisions were found. Sometimes neoplastic tissue was found directly underneath the serosal surface of the mesentery which was everywhere covered by typical mesothelium.

DISCUSSION

Since the tumor was composed of nonstriated muscular tissue and numerous thin-walled blood vessels, the diagnosis may be either leiomyoma angiomatosum or angiomyoma. The latter diagnosis implies that the blood vessels are neo-

FIG. 1. Thin-walled blood vessels separated by muscular tissue producing the aspect of cavernous tissue. Masson's tetrachrome stain. × 150.

FIG. 2. Tissue stained by Gomori's silver method. × 300

FIG. 3. Details of muscle cells. Masson's tetrachrome stain. × 600.

plastic and not just part of the stroma of a leiomyoma. An argument in favor of the latter concept may be found in the fact that in the leiomyomas of the uterus typical larger and smaller arteries are present in the connective tissue septa from which the muscle bundles obtain their blood supply, whereas in the present case arteries were completely absent from the tumor, being observed only outside the tumor tissue. The blood vessels in the tumor did not show special features, which could favor one or the other diagnosis.

A perusal of the different textbooks and treatises on tumors was not very helpful. Willis,[9] Ewing[3] and Boyd[2] do not mention the term angiomyoma. Robert Meyer[7] observed among several thousand uterine leiomyomas one case in which a diagnosis of myoma partum angiomatosum was made. His illustrations show that the blood vessels had a narrow lumen. The cells immediately surrounding the blood vessels were considered to be "unripe" muscle cells. Orsós[8] described as angiomyoma of the uterus a tumor composed almost exclusively of blood capillaries embedded in a small amount of muscular tissue. It is obvious that his case is quite different from ours in which muscular tissue predominated in some places and blood vessels in others. In view of the fact that it is impossible to prove with certainty that the blood vessels are neoplastic or are due to a malformation, we prefer the name, myoma angiomatosum. In this connection it must be mentioned that Ewing[4] believed he found striking evidence of the origin of tumor masses from blood vessels in a case of recurrent telangiectatic myoma of the broad ligament.

Since mitotic divisions were very rare in this case, and we always find numerous mitoses in malignant leiomyomas, we do not consider the tumor to be malignant. The finding of some large and atypical muscle cells has no great importance since such elements also occur from time to time in quite benign myomas of the uterus.

SUMMARY

From the mesentery of a 59 year old man a tumor was removed which on gross inspection showed areas resembling either myoma or angioma. On microscopic examination it was found to be composed of nonstriated muscular tissue and of thin-walled blood vessels, and in several places it resembled cavernous tissue. A diagnosis of leiomyoma angiomatosum was made. Neoplasms of this type seem to be rare, especially in the mesentery.

REFERENCES

1. Beitzke, H.: Ein metastasierendes Angiosarkom. Virchows Arch. f. path. Anat., **287**: 82–89, 1932.
2. Boyd, W.: Surgical Pathology. Ed. 6. Philadelphia: W. B. Saunders Co., 858 pp., 1947.
3. Ewing, J.: Neoplastic Diseases. A Treatise on Tumors. Ed. 4. Philadelphia: W. B. Saunders Co., 1941.
4. Idem., p. 235.
5. Foot, N. C.: Pathology in Surgery. Philadelphia: J. B. Lippincott and Co., 511 pp., 1945.
6. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie für Studierende und Ärzte. Ed. 9–10. Berlin und Leipzig: Walter de Gruyter, 990 pp., 1931.
7. Meyer, R.: Die pathologische Anatomie der Gebärmutter. In Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie. Band VII. Berlin: Julius Springer, p. 340, 1930.
8. Orsós, F.: Angiomyoma uteri. Zentralbl. f. Gynäk., **58**: 122–128, 1934.
9. Willis, R. A.: Pathology of Tumours. St. Louis: The C. V. Mosby Co., 992 pp., 1948.

CLINICOPATHOLOGIC CONFERENCE*

JOHNSON McGUIRE, M.D., AND PAUL N. JOLLY, M.D.

From the Cincinnati General Hospital, Cincinnati, Ohio

CLINICAL DATA

History. A 51 year old white housewife was admitted to the hospital on December 19, 1947, complaining of abdominal swelling and pain, and shortness of breath.

Dyspnea was exertional and, had been insidious in onset, first becoming evident in 1944. In October 1945 and January 1946, the patient had severe attacks of influenza, following which orthopnea and a nonproductive cough developed. These symptoms subsided, but the dyspnea persisted and became progressively worse. In March 1946, a roentgen film of the chest was said to have shown the heart to be normal in size and shape. Heavy lung markings radiated outward in all directions from both hila, gradually fading toward the periphery. No pleural or diaphragmatic abnormalities were seen. In October 1946, a sense of epigastric fullness and distress developed. No pain or vomiting occurred and no relation to meals was evident. In February 1947, the dyspnea and epigastric distress became so severe that the patient was confined to bed. She was hospitalized for study on February 16.

The patient had had scarlet fever in childhood and an appendectomy over 20 years ago. There had been two full-term deliveries, 25 and 18 years ago. She had received radium therapy for menorrhagia with subsequent cessation of menses four years ago. Impaired hearing had been present since her second pregnancy. She suffered from chronic sinusitis for many years and had iodized oil instilled into her sinuses two or three times a year for the past 12 years.

Physical examination after admission in February 1947 revealed the following significant findings: palmar erythema; harsh breath sounds with fine inspiratory rales audible at both lung bases; respirations, 24 per minute; the left border of cardiac dullness in the fifth interspace at the midclavicular line; cardiac rhythm regular, the sounds distinct and no murmurs audible; pulse rate, 116 per minute; liver edge just palpable but nontender; no edema.

The patient's course was uneventful, and by the fifth hospital day her pulse rate was 80 per minute at rest. It rose to 100 per minute after walking for one hour and during this time she had only slight dyspnea. She was discharged on the sixth day.

While in the hospital the following laboratory findings were recorded: red blood cells 5.8 million, hemoglobin 17.8 Gm., white cell count 8000, differential smear normal; urine 1 plus albumin with 10 to 12 white cells per high power field; serum protein 6.9 Gm. per 100 ml. with 5.8 Gm. of albumin; blood Kahn test negative; stool examination negative; venous pressure (right arm) 6.5 cm. of water at rest and 9.5 cm. after exercise; vital capacity 1.5 liters (45 per cent), circulation time with ether 7 seconds and with decholin, 27 seconds.

* Received for publication, April 12, 1949

Roentgen studies of the gallbladder and the gastro-enteric tract were normal. A chest film showed a cardiac width of 15.5 cm. and thoracic width of 28.5 cm. The heart was enlarged, particularly to the left. The left border was straight. Extensive linear densities in both lung fields appeared to radiate peripherally from the hila and were thought to represent peribronchial and interstitial fibrosis. The apices and bases were least involved. The hilar shadows were enlarged with a suggestion of right hilar adenopathy. The finds were quite similar to those observed a year earlier, except for an increase in extent. An electrocardiogram showed a rate of 90 with sinus rhythm; PR, .18 sec.; QRS, .04 sec.; P_1 and P_2 sharply peaked, measuring 4 and 5 mm.; P_3 and P_4 prominent but biphasic; QRS downward in lead I, upward in II and III and 8 to 10 mm. in height; RS biphasic in IV; T_1 upright, T_2 and T_3 inverted, T_4 biphasic.

In April 1947, x-ray therapy was given to the chest at another hospital. The patient was bedfast at home because of abdominal swelling until August 1947, when she was placed on a salt-poor diet and received periodic injections of mercupurin. Following this, she was able to lead a more active life. In November 1947, because of a questionable toxic reaction, mercupurin was discontinued. Abdominal swelling promptly recurred together with constant epigastric pain and nausea. This necessitated a return to the hospital on December 19, 1947.

Physical examination. The skin was slightly cyanotic and palmar erythema was present. Anterior cervical lymph nodes were palpable, bilaterally. Temperature was normal. Diminished resonance and breath sounds were heard at the base of the right lung and fine crepitant rales at the left base. Respirations were 30 per minute. The heart was slightly enlarged to percussion, the rhythm was regular and no murmurs were heard. Pulse was 90 per minute; blood pressure, 106/70. The liver was enlarged and tender, extending 2 fingersbreadth below the right costal margin. No ascites were detected.

Laboratory findings. Studies performed during the first few days revealed the following: 3+ albuminuria; icterus index, 8 units; serum bilirubin, 0.6 mg.; bromsulfalein test, no retention at the end of 45 minutes; cephalin-cholesterol flocculation, 3+ (24 hrs.); serum albumin, 4.0 Gm.; serum globulin, 2.75 Gm.; vital capacity, 43 per cent; venous pressure, 13 cm. water. The cyanosis could be relieved by 100 per cent oxygen.

Roentgen studies. There were no appreciable changes in the cardiac and pulmonary findings from those previously noted. Films of the hands, feet and pelvis exhibited no abnormalities of bones or joints.

Course in the hospital. A few days after admission a tricuspid systolic murmur was heard, but was recorded as insignificant. Because of dyspnea, cyanosis and progressive edema of the flanks, intramuscular injections of mercupurin were administered and satisfactory diuresis was obtained. However, the systolic murmur, rales, hepatomegaly and tachycardia persisted. Throughout her hospitalization the patient's symptoms fluctuated according to her mental state and were not related to any striking changes in the physical findings. Apprehension or irritation resulted in accentuation of the dyspnea, cyanosis, tachycardia, epigastric distress and elevation in venous pressure.

During the first two months in the hospital she was digitalized twice without

any improvement. Because of hoarseness of insidious onset, laryngoscopy was performed on January 23, 1948, and paralysis of the left vocal cord was discovered. A severe nonproductive cough developed. Potassium iodide was given intermittently but had to be discontinued because of signs of iodism.

During the hospital course, urine studies showed a maximum specific gravity of 1.010. There were persistent albuminuria, a few leukocytes and occasional granular casts. Blood counts were within normal limits. Repeated studies of sputum and gastric washings revealed no tubercle bacilli. (On one occasion it was reported that acid-fast bacilli were found.) Blood urea nitrogen determinations ranged from 14 to 28 mg. per 100 ml. Tuberculin tests were negative with .00002 mg. PPD, positive with .005 mg. PPD. Electrocardiograms were essentially the same as that already described. A tracing on June 5, 1948 showed P_2 to be 6 mm. and R_3 to be 25 mm.

On February 16 and 18, four attempts at basal metabolism determinations were made and the results ranged from +33 to +42 per cent. On February 24 she was placed on propylthiouracil, 50 mg. 4 times daily, and this was continued until May 12, when "dermatitis medicamentosa" developed. Considerable subjective improvement was noted, but little change was recorded in the physical state until June 22, when examination revealed a decrease in pulse rate to 72, reduction in edema of the flanks, dryness of the skin and absence of perspiration. These changes were thought to be on the basis of reduced thyroid function. Basal metabolic determinations on June 23 were +19 and −3 per cent.

For a period of two weeks in June the patient displayed apathy, lethargy, mental confusion, slurred speech, facial grimaces and muscular twitchings. These manifestations were thought to be on the basis of either hypothyroidism, hysteria, drug intoxication or a combination of these factors. The findings gradually subsided when all medications were stopped.

On July 15 she was discharged from the hospital to remain in bed at home. Examination on the day of discharge disclosed the following: Pulse, 95 per minute, harsh breath sounds audible over both lung fields, manifest cardiac enlargement, collapse of distended neck veins when the trunk was flexed to 40 degrees, liver edge 17 cm. below the xiphoid process and 6 cm. below the right costal margin, no ascites, dependent edema, or cyanosis. The patient was able to walk only a few steps without distress.

Course following discharge. There was a progressively downhill course. On September 25, the blood urea nitrogen was 49 mg. and the blood chlorides, 596 mg. Her temperature rose to 101 F. and on September 26, 1948 she expired.

CLINICAL DISCUSSION

Dr. Johnson McGuire. "We are confronted with a patient with extensive pulmonary infiltration of long duration with cor pulmonale, renal disease and possibly liver disease.

"Thirteen diagnostic possibilities present themselves to me in the differential diagnosis:

1. *Primary amyloidosis.* Amyloidosis may be classified as (a) primary amyloidosis, (b) amyloidosis secondary to infection, (c) amyloidosis associated with

multiple myeloma, and (d) amyloidosis causing the formation of nodular tumors. Fifty-three cases of primary amyloidosis have been reported.[6] In 27 of these there was clinical evidence of congestive failure and in 18 there was amyloid infiltration of the heart. In one case congestive heart failure resulted from infiltration by amyloid of the pulmonary blood vessels causing chronic cor pulmonale. In primary amyloidosis the liver, kidneys and spleen usually show minimal involvement while the lungs, skin, mucous membranes and lymph nodes may be extensively affected. The Congo red test has been helpful in corroborating the diagnosis when no tissue accessible for biopsy was available. This test was apparently not performed in the case under consideration.

2. *Pulmonary xanthomatosis.* The normocholesteremic type of essential xanthomatosis may produce chronic pulmonary fibrosis with lesions identical in appearance with those of the occupational type of pneumoconiosis. Since xanthomatosis of the lung has always been observed simultaneously with osseous xanthomata, lymph node and dural involvement and diabetes insipidus, this condition can probably be eliminated from the differential diagnosis in the patient under consideration.

3. *Interstitial fibrosis of the lungs.* In 1944 Hamman and Rich[4] described 4 fatal cases of acute diffuse interstitial fibrosis of the lungs of unknown etiology. These were characterized clinically by the usual manifestations of cor pulmonale and pathologically by "a remarkable proliferation of connective tissue that occurs within the walls of the alveoli". These cases lived for only a few months, and it is unlikely that our patient falls into this group.

4. *Obliterative bronchiolitis* has been described as a sequel to influenza, whooping cough, pneumonia and also occurs in Bright's disease. In the latter condition Ehrich and McIntosh[2] are uncertain as to whether this condition arises as a consequence of infection or of the toxemia associated with uremia.

5. *Pulmonary arteriosclerosis* and pulmonary hypertension usually produce some increase in the density of the hila but the peripheral lung fields, unlike those in this patient, are usually clear. Thus it seems unlikely that we must consider Ayerza's disease.

6. *Pneumoconiosis* is a pulmonary disorder characterized by chronic fibrous reaction of the lung due to the prolonged inhalation of injurious mineral dusts, such as granite, flint and asbestos. There is no evidence that this patient was ever exposed to such agents and while the x-ray finding is compatible with such a disorder, I shall dismiss it from consideration.

7. *Carcinoma of the lung.* The long duration, freedom from chest pain and hemoptysis, and complicating pulmonary infection as well as the x-ray appearance make such a diagnosis unlikely.

8. *Lymphoma.* Jackson and Parker[5] have described 3 forms of Hodgkin's disease: (a) the relatively benign paragranuloma, (b) the granulomatous form, and (c) the sarcomatous type. On rare occasions the lungs alone may be involved by Hodgkin's disease but this condition is so rare as a diffuse disease, and in this patient the duration of life so long, that such a diagnosis seems improbable.

9. *Pneumomycosis.* Since fungus infections of the lungs may progress extremely slowly and may be characterized by the development of extraordinary fibrosis, this condition cannot be dismissed but cannot be confirmed unless fungi were found in the microscopic sections. Such fungi could have been molds, yeasts or streptothrices.

10. *Tuberculosis* without more localization in the lung, without fever or evidences of sepsis, with a weak tuberculin reaction and with the x-ray pattern described, seems highly improbable.

11. *Periarteritis nodosa and other 'collagen diseases'.* Extensive involvement of the lung has been observed in the course of a number of the 'collagen diseases'. The absence of eosinophilia, skin rash, hypertension and more advanced renal disease make such a clinical diagnosis unwarranted.

12. *Sarcoidosis* must be seriously considered as the underlying disease in this patient. The manifestation of the disease may be divided into 4 clinical types: (a) Sarcoids of the skin as described by Boeck, (b) uveoparotid fever, (c) the glandular type with involvement of superficial or intrathoracic nodes, and (d) the type with primary involvement of the lung which may closely resemble pulmonary tuberculosis. The latter may prove to be the correct diagnosis, but I am disappointed about the absence of lymph node enlargement of more conspicuous degree than was encountered in this case.

13. *Lipoid pneumonia.* So far as I can ascertain, lipiodol, unlike mineral oil, has not been shown to produce diffuse pulmonary fibrosis. It has been extensively employed by direct instillation into the lung and diffuse fibrotic lesions have not, to my knowledge, been demonstrated. It seems highly improbable that its instillation in the sinuses was responsible for the pulmonary lesion here, although I confess it remains a possibility.

"In summary, I have endeavored to describe several types of pulmonary lesions which may have elevated the pulmonary arterial blood pressure in this patient and produced cor pulmonale. During life, I had the opportunity of examining the patient with several distinguished clinicians and a definite diagnosis was impossible. It may be that the pathologists likewise have had difficulty in explaining the lesion which caused the x-ray shadows. In other words, I should like to see convincing evidence advanced by the pathologists of a specific etiologic agent or entity which caused the illness and death of this patient, and I would not be surprised if the causation of the pulmonary fibrosis cannot be determined. My diagnosis is pulmonary fibrosis of unknown etiology, generalized arteriosclerosis, arterionephrosclerosis, cor pulmonale and chronic passive congestion of the liver."

Dr. M. A. Blankenhorn. "Dr. Felson, would you tell us what you have gathered from the films?"

Dr. Benjamin Felson. "H. Felson and Heublein[3] have listed approximately 80 causes for diffuse pulmonary densities of the type seen in this case. Most of the common causes, such as miliary tuberculosis, lymphogenous spread of metastatic carcinoma, and bacterial and viral types of pneumonia can be eliminated in this patient because of the absence of change during the prolonged period of

roentgen observation. A number of less common causes can be eliminated on clinical grounds. These include congenital cystic disease and cystic disease associated with tuberous sclerosis, xanthomatosis of the lungs, hemosiderosis of the lungs as seen in mitral stenosis, syphilis, periarteritis and erythema nodosa and scleroderma.

"This leaves a few conditions which cannot be eliminated from consideration from a roentgenological standpoint:

(1) The fungus disorders must receive serious consideration. A diagnosis of mycotic infection of the lungs, however, requires laboratory confirmation.

(2) Pulmonary alveolar adenomatosis (jagziekte) or so-called Montana sheep disease is being encountered with more and more frequency in human subjects. We have seen roentgenograms in 5 such patients in the past two years and in 2 of them the appearance closely resembled that of the present case. The lack of progression and the absence of large quantities of frothy sputum speak against this condition.

(3) The cases of acute diffuse interstitial pneumonitis described by Hamman and Rich[4] ran a much more rapid course than the present case, but the roentgenograms were quite similar.

(4) Pulmonary sarcoidosis frequently shows the appearance seen in this patient and the evidence of hilar lymphadenopathy favors this condition.

(5) Lipoid pneumonia can give this appearance, and we have seen such a case due to mineral oil. In view of the history of frequent instillation of lipiodol into the nasal sinuses, the possibility of this substance producing the pulmonary changes must be given serious consideration. I have heard of one case in which iodochloral produced interstitial fibrosis in the lungs. Amberson[1] described pulmonary roentgenograms mistaken for pulmonary tuberculosis, bronchiectasis and associated pulmonary fibrosis which actually proved to be fibrosis due to lipiodol. One of his cases closely simulated what we see here.

"The roentgen diagnosis in this case is, therefore, diffuse pulmonary fibrosis associated with miliary nodules, probably due to lipiodol or sarcoid."

Dr. William F. Ashe. "Did the patient work in any factory during the war?"

Dr. Blankenhorn. "I am certain she did not."

Dr. Felson. "Had she any contact with beryllium as in the fluorescent light industry?"

Dr. Blankenhorn. "To my knowledge there was no industrial contact whatever. She had been a hospital nurse prior to her marriage and was occupied as a housewife thereafter."

Dr. Eugene B. Ferris. "Haven't some cases of lipoid pneumonia been attributed to iodized oil?"

Dr. Felson. "Yes, several such cases, in addition to those I've cited, have been reported, although I don't recall how well authenticated they were."

Dr. Joseph N. Ganim. "Were the lymph nodes noted on the first entry large enough to indicate a biopsy?"

Dr. Blankenhorn. "They were not. The only biopsy made in this patient was upon a redundant abdominal scar and this showed only a cicatrix."

Dr. Robert J. Ritterhoff. "How much roentgen therapy had been given to the chest in this case? What is the tolerance to x-ray of the lung?"

Dr. Felson. "We do not know how much therapy was given, since the treatment was carried out at another hospital. When we give therapy to a superficial lesion of the chest we usually try to direct treatment tangentially in order not to affect the lung. Pulmonary fibrosis has been reported as occurring following dosages under 1000 roentgens."

Dr. Ritterhoff. "Do you think the roentgen therapy aggravated the underlying pulmonary disease?"

Dr. Felson. "No, I don't believe so since films taken before and after therapy show no evidence of alteration."

Dr. Ritterhoff. "The history relating to the terminal phase of the illness is rather scanty. Was this, and for that matter the evidences of mental confusion, the result of interference with blood gas exchange?"

Dr. Blankenhorn. "She frequently suffered from anoxia, and I believe that this colored many of the clinical manifestations."

Dr. Dale P. Osborne. "Mental confusion was a prominent feature during the terminal course. The liver remained large and tender but there was no ascites. Her temperature rose but slightly, and there was no significant change in her general condition."

PATHOLOGIC FINDINGS

Dr. Paul N. Jolly. "The autopsy, limited to an abdominal incision, was performed at a funeral home after the body had been embalmed. Edema was absent. The superficial lymph nodes were not enlarged. The skin displayed no abnormalities except for a well-healed laparotomy incision. The peritoneal cavity contained 200 or 300 cc. of reddish tan fluid, thought to represent embalming fluid. The thoracic organs were removed through the diaphragm.

"The *lungs* were rather small but were moderately increased in weight. Fibrinous pleuritis was seen over the right middle lobe, and the left upper lobe displayed considerable superficial emphysema. The lungs cut with increased resistance to reveal firm, slightly granular, mottled dark gray tissue. A 1 x 2 cm. abscess filled with creamy purulent exudate was present in the right middle lobe immediately beneath the pleura. In the tissue around this abscess were many confluent firm, yellowish miliary nodules. Occasional similar nodules were scattered through the other lobes. The apical portion of each lung was honeycombed with many small cystic spaces measuring up to 3 mm. in diameter. The intervening tissue was streaked with an irregular network of anthracotic fibrous tissue, the strands of which measured up to 1 mm. in thickness. The bronchial mucosa was slightly hyperemic, smooth, and covered with a small amount of tenacious mucus. The pulmonary arterial tree was dilated and presented prominent yellow streaking of the intima. There was no evidence of thrombosis. The tracheobronchial lymph nodes were enlarged, the largest measured 3 x 2 x 0.5 cm.

"Microscopically the lungs revealed a marked, diffuse interstitial fibrosis.

Within many fibrous zones were collapsed alveoli lined by cuboidal epithelium. Other air spaces were markedly dilated, the lining varying from squamous to cuboidal cells. The bronchi were dilated and erosion of their cartilaginous plates was frequently observed. A chronic active granulomatous inflammation was manifested by foci of isolated or confluent poorly defined tubercles. Giant cells were frequent in the tubercles and occasionally were seen isolated in the fibrous tissue. Rarely cytoplasmic inclusions of the type described by Schaumann[7] and Wolbach[8] were seen in the giant cells.

"Superimposed on this chronic sclerosing process was an acute lobular pneumonia with foci of macroscopic and microscopic abscess formation. In the areas of acute pneumonia, moderate to marked inflammation of the thickened fibrotic septa were seen. Marked thickening of the intima of the larger branches of the pulmonary artery were presumably due to pulmonary hypertension. Several small foci of anthracosilicosis were also demonstrated.

"In an effort to reconstruct the pathogenesis of the pulmonary fibrosis, special stains were used. Sections stained for elastic tissue showed the elastic tissue fibers to be irregularly fragmented and embedded in the collagenous scar, indicating that the framework of the lungs was severely disrupted and that the fibrosis was not a mere additive feature. Fat stains on the lungs revealed only an occasional area where fatty globules were trapped in the scar tissue. Small droplets of fat were also encountered in phagocytes within the alveoli. In no instance were they of sufficient prominence to indicate a relationship with the development of the process.

"The tracheobronchial *lymph nodes* also revealed a chronic granulomatous inflammation with more signs of activity than in the lung but with less fibrosis. Tubercles, usually poorly defined and occasionally consisting only of giant cells, were seen throughout the sections. Conchoidal Schaumann bodies staining either red or blue, were more numerous in the lymph nodes than in the lung. An occasional Wolbach asteriod was encountered within the giant cells. In the lymph nodes, deposits of fat were occasionally encountered in the thickened capsule and smaller droplets were rarely seen in some of the tubercles. Examination of sections of the lungs and lymph nodes under polarized light failed to reveal any material which could be considered of etiologic significance. Bacterial stains, Gram and acid-fast, revealed no organisms which were considered pertinent. Amyloid stains failed to reveal amyloid in the lungs or lymph nodes.

"The *heart* was large, was estimated to weigh 450 Gm. and showed marked hypertrophy and dilatation of the right ventricle and atrium. The interventricular septum bulged into the left ventricle due to dilatation of the right ventricle. The trabeculae carneae of the right ventricle were hypertrophied and flattened. Mural and valvular endocardial surfaces appeared normal. The myocardium was a uniform dark red. The coronary arteries and aorta contained scattered, slightly elevated intimal plaques.

"The *liver* was of average size with a normal-appearing capsular surface. Sections revealed an accentuated lobular pattern with the lobules projecting slightly above the cut surface. The central veins were prominent. Histologically there

was marked acute and chronic passive hyperemia with marked central zone necrosis. The portal triads were somewhat more fibrous than usual. The *spleen* appeared normal grossly, and microscopically revealed only marked acute passive hyperemia. Grossly the *kidneys* appeared to be of normal configuration, and on section revealed normal markings in the cortical and pyramidal zones. Microscopically there was a marked toxic nephrosis with loss of the nuclear staining in many of the epithelial cells of the tubules. Some glomeruli showed early signs of a focal glomerulitis in addition to the changes associated with acute passive hyperemia. Except for scattered petechiae in the gastric mucosa, the remainder of the abdominal and pelvic viscera were essentially normal."

ANATOMIC DIAGNOSIS

Chronic pneumonitis with marked interstitial pulmonary fibrosis, etiology undetermined.

Pulmonary emphysema and bronchiectasis.

Terminal lobular pneumonia with abscess formation.

Chronic granulomatous inflammation of tracheobronchial lymph nodes.

Cardiac hypertrophy, predominantly right ventricle (chronic cor pulmonale).

Passive congestion, chronic, abdominal viscera with centrolobular necrosis of the liver (evidence of right-sided heart failure).

Acute toxic nephrosis, terminal.

GENERAL DISCUSSION

A student. "What was the cause of the laryngeal nerve paralysis?"

Dr. Jolly. "I believe that this was probably due to pressure by the enlarged mediastinal nodes."

Dr. Ferris. "May an oily substance produce such a lesion and not be evident in the autopsy material?"

Dr. E. A. Gall. "Yes, I think so. It would depend a great deal upon the fineness of the globules and their dispersion. Over a period of years they may well have been phagocytized and carried away from the original loci."

Dr. Blankenhorn. "Have such occurrences been reported?"

Dr. Rittenhoff. "I do not recall reading of such cases."

Dr. Blankenhorn. "Could sarcoid have caused this process and the characteristic lesion then be obscured by involution and scarring?"

Dr. Gall. "I think it could, but there are certainly no characteristic stigmata remaining."

Dr. Blankenhorn. "Was the hilar density on the right due to a prominent pulmonary artery or to enlarged lymph nodes?"

Dr. Jolly. "To enlargement of lymph nodes."

A student. "Were there any lesions in the adrenals?"

Dr. Jolly. "No, there were none."

Dr. H. Felson. "Could the change here be a sequela of such a lesion as Hamman and Rich described?"

Dr. Jolly. "Their cases were characteristically acute. It is conceivable, of

course, that an acute process of that nature may have continued in a surviving patient and resulted in such a picture as we see here. As Dr. McGuire astutely stated, we can only speculate as to the original causative agent or process. There is no way in which we can definitely identify it at this time."

REFERENCES

1. Amberson, J. B., Jr., and Riggins, H. M.: Lipiodol in bronchography; its disadvantages, dangers and uses. Am. J. Roentgenol., **30:** 727–746, 1933.
2. Ehrich, W., and McIntosh, J. F.: The pathogenesis of bronchiolitis obliterans; observations in cases of Bright's disease. Arch. Path., **13:** 69–79, 1932.
3. Felson, H., and Heublein, G. W.: Some observations on diffuse pulmonary lesions. Am. J. Roentgenol., **59:** 59–81, 1948.
4. Hamman, L., and Rich, A. R.: Acute diffuse interstitial fibrosis of lungs. Bull. Johns Hopkins Hosp., **74:** 177–212, 1944.
5. Jackson, H., Jr., and Parker, F., Jr.: Hodgkin's disease; general considerations. New England J. Med., **230:** 1–8 and **231:** 35–44, 1944.
6. Lindsay, S.: The Heart in Primary Systemic Amyloid Disease. Modern Concept of Cardiovascular Disease. American Heart Association, Vol. 17, No. 6, 1948.
7. Schaumann, J.: On the nature of certain peculiar corpuscles present in tissue of l mphogranulomatosis benigna. Acta med. Scandinav., **106:** 239–253, 1941.
8. Wolbach, S. B.: A new type of cell inclusion, not parasitic, associated with disseminated granulomatous lesions. J. M. Research, **24:** 243–257, 1911.

EDITORIAL

The Training of Clinical Pathologists

Clinical pathology is facing the accusation that it is not prepared to train residents adequately in this specialty. Admittedly it is easier to arrange a satisfactory program in pathologic anatomy. Many hospitals can provide a good program in autopsies and surgical pathology, but are embarrassed when it comes to providing a formal curriculum in clinical pathology. There are many hospitals with a profitable laboratory which do not provide an adequate professional staff in clinical pathology and consequently the residents are forced to receive most of their formal instruction from the technicians or from the overworked pathologist.

Some teaching hospitals fail or refuse to recognize the importance of clinical pathology as part of the residency training program, despite the fact that the average pathologist is a hospital pathologist and devotes approximately three quarters of his time to clinical pathology. Many residents have been unhappy to realize suddenly at the completion of their training that this important phase of their specialty had been neglected. In many instances it has been necessary for these young men to secure supplemental training.

The greatest need in this field today is for hospital pathologists, and yet residents and fellows are not being thoroughly trained. Their future professional career is thereby embarrassed and many small and medium-sized hospitals are left in desperate need of pathologic service. It would appear that pathologists and departments of pathology have been unwise in ignoring clinical pathology.

In many teaching institutions the teaching of pathology does not include clinical pathology, the director of the department being willing that other specialists or non-medical people direct the practice of laboratory medicine. The defense of such a practice might be that these departments of pathology do not possess the facilities to train men in clinical pathology. Often this is a helpless and convenient attitude, one which could be corrected by departmental and inter-departmental re-organization or by establishing affiliations with the basic science divisions.

The practice of pathology does not consist merely of the practice of hematology or chemistry or pathologic anatomy or microbiology or any other highly specialized branch of laboratory medicine. It consists of a broad application of knowledge, skill and experience in the whole specialty. From the teaching viewpoint over-emphasis of any one of the subspecialties is harmful. Unless pathologists can see that it is mandatory for broad concepts to be adopted and executed, it seems inevitable that clinical pathology will undergo dissolution and absorption.

Detroit Osborne A. Brines

SELECTED ABSTRACTS

The Mechanism of Petechial Hemorrhage Formation. J. G. HUMBLE. Blood, 4: 69–75, 1949.

The skin capillaries of 17 patients with petechial hemorrhages of various etiologies were studied by means of the Leitz "ultrapak" microscope. Regardless of the primary cause, hemorrhage was always localized to the arteriolar end of the arteriolocapillary junction at the site of sudden dilatation to form the capillary loop. Intracapillary pressure is higher at this point than elsewhere in the loop, and it is in this region that fluids normally leave the vessels for the tissues. Selective damage to the arteriolocapillary junction is proposed to explain many types of hemorrhagic syndromes.

The fate of the extruded red blood cells, both as to manner and time of removal was seen to be different in "primary" and secondary thrombocytopenias, scurvy, and anaphylactoid purpura.

Cleveland BEN FISHER

Acquired Afibrinogenemia in Pregnancy. W. C. MOLONEY, W. J. EGAN and A. J. GORMAN. New England J. Med., 240: 596–598, 1949.

A 25 year old gravida ii entered the hospital because of lower abdominal pain and spontaneous bleeding from the gums. She had been well throughout her pregnancy except for occasional abdominal pains. The patient's past history and the family history were negative for abnormal bleeding. However, she had noticed slight bleeding from the gums during her other pregnancy, two years previously. The clotting time (modified Lee-White) was 16 hours, and only a poor gel formed. The bleeding time (Duke) was 14 minutes and the capillary fragility test was strongly positive. The platelets were normal. Prothrombin time (Quick) was 42 seconds, with a 14-second control. Addition of thrombin to the blood did not induce clotting *in vitro*, indicating a deficiency of fibrinogen.

Because of the increasing bleeding a cesarean section was performed. As soon as the plum-colored hemorrhagic uterus was removed, the bleeding seemed to cease. No further bleeding was noted and the subsequent hospital course was uneventful. Examination of the patient's blood coagulation mechanism was negative during the rechecks for several months afterward. Chemical analysis of a specimen of oxalated blood drawn before the operation showed complete absence of fibrinogen. After the operation, fibrinogen levels ranged from 0.35 to 0.50 Gm. per 100 ml. The authors attempt to explain the syndrome by the presence of a fibrinolysin or fibrinogenolysin. These could not subsequently be demonstrated.

This case emphasizes the importance of investigating the fibrinogen levels in patients with prolonged clotting times and low prothrombin levels.

BEN FISHER

Balantidium Infection in the Dog. W. S. BAILEY and A. G. WILLIAMS. J. Am. Vet. M. A., 104: 238, 1949.

This is apparently the second reported case in the dog. The animal, a 3 or 4 year old male Pointer, had eaten the intestines of a hog that had been killed on a farm. Several days later the dog began to have a bloody diarrhea, and soon thereafter, large numbers of *Balantidium coli* were found in the stool. Following treatment with sodium sulfamethazine, the dog recovered (about four weeks after onset of diarrhea) and the organisms disappeared from the stool. It is possible that the infection was self-limiting.

BOOK REVIEWS

Neoplasms of Bone and Related Conditions, Their Etiology, Pathogenesis, Diagnosis and Treatment. By BRADLEY L. COLEY, M.D., Attending Surgeon, Bone Tumor Department, Memorial Hospital for Cancer and Allied Diseases, Assistant Professor of Clinical Surgery, Cornell. 765 pp., 622 figs., 53 tables. $17.50. New York: Paul B. Hoeber, Inc., 1949.

In *Neoplasms of Bone* there are brought together in one volume the clinical diagnostic, roentgenologic, pathogenetic and therapeutic facts concerning not only the true bone tumors but also the various and sundry affections of bones and related tissues which must enter into the differential diagnosis of bone lesions. In compiling the present-day knowledge of this complex field the author has drawn amply from his twenty-five years' experience in the Bone Tumor Department of Memorial Hospital. An instructive introduction includes a classification in keeping with that of the Bone Tumor Registry, a discussion of etiologic factors and a comprehensive survey of the diagnostic procedures suggested in suspected cases of bone tumor. History, physical and roentgenologic examination, laboratory studies, the various technics of biopsy, even the methods of preparation of the specimen are carefully described. Differential diagnosis is emphasized, generally at the outset, with excellent tables outlining the cardinal points. As each specific lesion is discussed the differential diagnoses are again individually assessed. Ten additional sections make up the assembly. The section on benign tumors devotes a chapter each to osteoma, osteoid osteoma, nonosteogenic fibroma, chondroma, chondrodystrophia calcificans congenita, benign chrondroblastoma of bone, myxoma, hemangioma, solitary cyst of bone, fibrous dysplasia, xanthoma, giant cell tumor, adamantinoma, chordoma, and sarcoma as a late development in benign lesions of bone. The section on primary malignant tumors is equally comprehensive. Moreover, separate sections are devoted to tumors involving bone by extension, metastatic tumors involving bone, tumors of bone in special localities, and inflammatory, toxic and metabolic lesions of the skeletal system that may simulate neoplasms of bone.

An example of the many-sided approaches to each subject is the fact that in almost every chapter there is a discussion of age incidences of the individual lesion. In addition, however, there is a separate discussion of tumors of bone in childhood. Again, whenever applicable to individual lesions, the value of the blood chemical analysis is discussed; but the book contains in addition a thorough chapter on blood chemistry in respect to bone metabolism and differential diagnosis. The discussion of each lesion usually includes the definition and history of the lesion, a survey of the various theories of its etiology, the symptoms and physical signs, the gross and microscopic pathology, the differential diagnosis, evaluation of the various surgical and roentgenologic treatments suggested in the literature as well as the author's recommendations, the technics of treatment and finally the prognosis. But for completeness, large sections have been added, devoted entirely to surgical treatment, radiation therapy and constitutional therapy. The pathologist may be disappointed at first because the book is not an atlas of microscopic pathology; however, the completeness of the life history of each of the long list of bone lesions discussed will more than repay his application to this book. In the preface, the author in all fairness mentions the text books on pathology which best supplement his work. Even so there is an illustration for almost every page.

The orthopedist, the radiologist, the general surgeon and the general practitioner will find their respective interests interestingly and as completely covered as present knowledge permits. The print is large and easy to read, and the quality of the paper and of the binding is of the high standard we have come to expect from this publisher.

Detroit

JOHN W. REBUCK
FRANK W. HARTMAN

A Textbook of Histology. Ed. 5. By ALEXANDER A. MAXIMOW, Late Professor of Anatomy, University of Chicago; and WILLIAM BLOOM, Professor of Anatomy, University of Chicago. 700 pp., 562 figs. $8.50. Philadelphia and London: W. B. Saunders Company, 1948.

The fifth edition of Maximow and Bloom brings this standard textbook up to date. Through its several editions effort has been made to bring the book more and more into line with the supposed needs of the present day medical student. There are those, including the reviewer, who believe that none of the later editions quite equaled the first. It may be that we liked it for the wealth of information and the excellent description which it contained or maybe because it was an excellent reference text. It may be that the newer editions are better for medical students. Certainly the great number of illustrations, most of them good, make things easier for students. In any event, they all seem to like the profusely illustrated text.

The revised section on protoplasm has been excellently handled by William Doyle. He deserves special commendation for his discussion of submicroscopic organization. Most of the recent information is condensed and clearly set forth for medical student consumption. The inclusion of some of the results of phase difference microscopy is timely, but, unfortunately, little or nothing is said about the process or what its use may be expected to reveal. The same applies to the mention of electronmicrography. The reviewer is of the opinion that much is to be gained by informing medical students about some of the ways of research. By this means their interest is more readily captured and retained. Frequently a gadget may serve to lead the student into inquiry on his own. It should be remembered that many great microscopists first became intrigued with the instrument.

In general the balance of the book has been improved. The amount of space devoted to blood and its origin has been reduced considerably. It has always amazed the reviewer that most of the textbooks in histology devote an inordinate amount of space to such things as spermatogenesis and so little to others which would seem to be significant. This one is no exception with roughly 9 pages on spermatogenesis and 11 on pancreas, 8 on the salivary glands, 2 on the parathyroids, and 6 on the adrenals. Even if one considers histology to be *only* a preparation for pathology, the space investment would seem to be disproportionate.

The new format with its double-columned pages makes for easier and more rapid reading. The references to the literature are sufficient in amount, and the index is excellent. The amount of fine print has, if anything, been increased. The logical background for this is, to the reviewer, a complete mystery. Perhaps these are things the young medical student shouldn't know; certainly no more effective way could be devised to conceal them.

Detroit GORDON H. SCOTT

Virus Diseases of Man. Ed. 2. By C. E. VAN ROOYEN, M.D., D.Sc. (Edin.), M.R.C.P. (Lond.), Research Member and Professor of Virus Infections, Connaught Medical Research Laboratories and School of Hygiene, University of Toronto, Formerly Sir Halley Stewart Research Fellow and Lecturer in Bacteriology, University of Edinburgh; and A. J. RHODES, M.D., R.F.C.P. (Edin.), Research Associate and Associate Professor of Virus Infections, Hospital for Sick Children, Toronto, Formerly Lecturer in Bacteriology, University of Edinburgh, and London School of Hygiene and Tropical Medicine, University of London. 1202 pp., 43 figs. $22.50. New York: Thomas Nelson and Sons, 1948.

As stated in the preface, the purpose of this book is to provide a volume of reference for both the laboratory worker and clinician interested in the field of human and animal virus infections. In this aim the book succeeds admirably. The first chapters deal with the fundamentals of methods of study and their interpretations, and includes excellent examples and illustrations. In the individual chapters, procedures are also discussed as they refer to the particular virus and disease problem.

In its arrangement the book tends to classify virus diseases on the basis of broad simi-

larities but in some respects the classification is quite irregular in that certain virus infections, as that of West Nile virus, are included under arthropod-spread infections while the encephalitides of the temperate zones are listed as encephalitis. Although there are inequalities in the emphasis given to some diseases, the volume presents an excellent balance and uniformity of presentation. The literature is thoroughly incorporated into the context of discussion and various points of view are adequately presented. In these respects the book furnishes excellent source material for orientation and further exploration by readers of all levels of training.

Each infection is presented in its clinical, epidemiologic, preventive and etiologic aspects, with the latter considering the characteristics of the agent and the serologic or other diagnostic reactions. Essentially all the significant virus diseases of man are included. The bibliographies are excellent and the index is satisfactory.

This is an excellent book, strongly recommended to students of infectious disease at any level. It's worth the price.

Ann Arbor, Michigan THOMAS FRANCIS, JR.

Acta Haematologica. International Journal of Hematology. Edited by E. MEULENGRACHT of Copenhagen, K. ROHR of Zurich and G. ROSENOW of New York. Basel, Switzerland and New York: S. Karger.

The purpose of the hematologic periodical, *Acta Haematologica*, is to publish articles concerning the blood and its disorders, with particular emphasis on its international scope, by acceptance of articles in three languages, English, French and German.

The interest in hematology has been greatly intensified in recent years. In addition to the clinical and morphologic methods of study, a functional, dynamic approach to the various problems has been developed. The practical importance of serologic data for the understanding and the rational treatment of hemolytic diseases has been recognized. The application of physico-chemical methods has opened entirely new fields of research.

While much of this newer knowledge had been published in journals with diversified interests in the field of medicine, the demand to have an international journal not limited to one language was frequently heard, particularly since the Folia Hematologica was discontinued.

Acta Haematologica is such a periodical; planned for the exchange of thoughts and findings of hematologic research in all countries. The journal is intended to forge a scientific link between the hematologists of the old and new world.

Since the inception of *Acta Haematologica* one year ago, articles have been published dealing with clinical hematologic problems as well as with morphology and pathophysiology of the blood and the blood-forming organs.

Besides original articles, reviews and surveys of the literature have been published on hematology, particularly of papers which are not readily accessible, as those in Spanish and the Scandinavian languages. Book reviews and information of general interest concerning hematologic activities in other countries also appear regularly in this journal.

Acta Haematologica was issued in the first year quarterly but will now be published bimonthly to form an annual volume of about 400 pages. Every article, whether published in English, French or German, contains a summary in each of the three languages.

The Editorial Board is represented by members from most of the countries of Europe and from North and South America.

Chicago LOUIS R. LIMARZI

NEWS AND NOTICES

Letter of Correction

April 1, 1949

To the Editor:

In a report[1] outlining a method for the fractionation of serum proteins, we recommended that in the step employed for separating serum into its albumin and total globulin components, the span-ether albumin-sodium sulfite solution be shaken vigorously for 30 seconds prior to centrifuging (Step 3, C). However, we have found that shaking for this period of time will lead to abnormally low serum albumin values, this being due to a loss of albumin during the shaking process. The effect of various periods of shaking, as outlined in Table 1, demonstrates that increased duration of shaking of a pure albumin-sodium sulfite mixture

TABLE 1

Per Cent of Albumin Remaining in an Albumin-Sodium Mixture Treated with Ether as Indicated

SHAKING		INVERTING	
Time in Seconds	Protein Concentration (% Remaining)	Number of Times	Protein Concentration (% Remaining)
0	100	0	100
3	100	5	99
5	99	10	98
10	97	20	97
20	93		
30	88		

with span-ether or plain ether leads to progressive loss of albumin. On the other hand, this loss is negligible with simple gentle inversion, regardless of the number of inversions.

From the data above, it became obvious that the directions given in our paper[1] under Step 3, C which call for vigorous shaking of the serum sulfite span-ether mixture for 30 seconds should be changed to read *invert gently about 5–10 times.*

We are grateful to Dr. M. Saito of St. Luke's Hospital, Tokyo, Japan, and Dr. Isadore Pitesky of the University of Illinois Medical School, Chicago, Illinois, for calling this matter to our attention.

Department of Biochemistry
Michael Reese Hospital
29th Street and Ellis Avenue
Chicago 16, Illinois

Clarence Cohn
William Q. Wolfson

REFERENCE

1. Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F.: Studies in serum proteins; a rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 1.0 ml. of serum. Am. J. Clin. Path. **18:** 723–730, 1948.

TECHNICAL SECTION

RAPID MEASUREMENT OF SERUM TOTAL BASE AND ESTIMATION OF SERUM SODIUM

An Improved Conductivity Assembly*

F. WILLIAM SUNDERMAN, M.D.

From the University of Texas Graduate School of Medicine and the M. D. Anderson Hospital for Cancer Research, Houston, Texas

A method for determining total base in serum by means of conductivity measurements has been described in previous papers.[3, 4, 6] It has been shown that the mean percentile deviation of serum total base obtained by conductivity measurements from those by chemical analyses is approximately 1.5 per cent. In this paper is presented an improved design of both the bridge and cell assembly for making conductivity measurements in serum. In addition it will be pointed out that the concentration of serum sodium can be estimated from such measurements with sufficient accuracy for most clinical purposes.

The improved bridge adapted for measurements in serum† operates on a 110 volt, 60 cycle alternating current. The circuit is that of a modified Wheatstone bridge driven by a 1000 cycle electronic tube oscillator 6D6. The bridge unbalance voltage is fed to a single stage amplifier, 6D6, coupled to a cathode ray tube, 6E5 (magic eye) which serves as a null-point indicator and provides reliable readings unaffected by vibration. The instrument includes compensating resistances to correct for changes in temperature and cell constants. The temperature compensator corrects for deviations from 25 degrees when the range of temperature is between 23 and 27 C. It has been shown previously[6] that in this range the average temperature coefficient for serum is 2.1 per cent per degree centigrade. The cell constant compensating resistance is designed to correct for changes in cell constants ranging between 8.5 to 13 reciprocal cm. Employing the temperature and cell constant resistances, it is possible to obtain direct readings of the specific conductivity of serum expressed as mhos (reciprocal ohms) $\times 10^{-3}$ at 25 C.

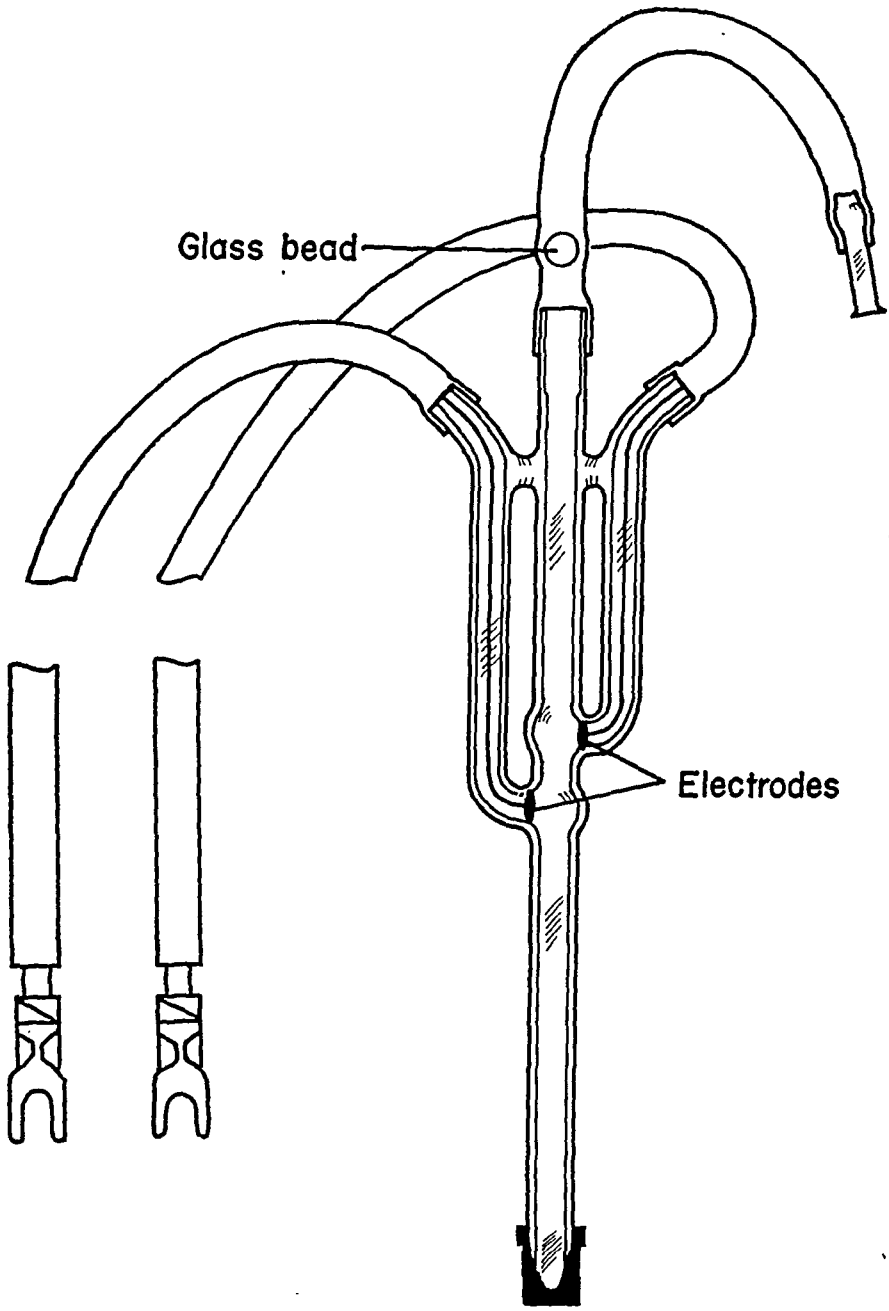
In the conductivity pipet cell previously described, the platinized electrodes extended horizontally into the lumen of a stopcock pipet. When serum is introduced into this cell, bubbles occasionally accumulate around the electrodes and dislodging them is frequently time-consuming. In addition, grease from the stopcock of the pipet is occasionally introduced into the cell and may lead to errors in measurement. The new conductivity cell (Fig. 1) has been designed to prevent accumulation of bubbles and the introduction of grease.

The electrodes in the new cell are parallel and flush with the walls of the

*This work was supported in part by a grant from the Office of Naval Research.

Received for publication, April 11, 1949.

†The complete conductivity assembly can be secured from the Arthur H. Thomas Company, Philadelphia, Pa.



SERUM CONDUCTIVITY CELL

FIG. 1.

pipet. This arrangement permits a free flow of serum through the lumen and avoids accumulation of bubbles around the electrodes. The new cell is more economical of serum than the former one,—the capacity being decreased to 0.5 ml. The over-all length of the cell from the tip to the constriction above the

upper electrode is approximately 10 cm. This length permits the use of a household tumbler as a water bath for immersing the cell. It is convenient to have about 20 inches of flexible rubber tubing attached to the upper end of the pipet, as shown in the diagram. The insertion of a small glass bead into the tubing serves as an efficient, economical valve. The bead can be located readily by the bulge in the tubing. With this design of cell no stopcock is necessary.

The pipet cell is filled by drawing in the serum, simultaneously pulling the rubber tubing to the side, away from the glass bead. After the serum is withdrawn into the cell a rubber cap is applied over the tip of the pipet and the pipet is placed into a tumbler of water the temperature of which has been manually adjusted between 23 and 27 C.

PROCEDURE

The serum to be measured is introduced into a dried cell. Drying is secured by thoroughly rinsing with absolute alcohol and drawing, not blowing, air through the cell. After serum is introduced and the temperature allowed to come to equilibrium (in approximately one minute) the temperature and cell constant compensators are adjusted to the correct values and the bridge is balanced by turning the dial control until the "magic eye" indicator opens completely. A balanced position is obtained when the dark portion of the signal reaches the maximum opening. Ordinarily only the portion of the scale between 9 and 15 mhos $\times 10^{-3}$ will be used for the serum measurements.

As early as 1898, Bugarsky and Tangl[1] pointed out that conductivity measurements were diminished in the presence of protein. Since that time various workers have attempted to apply corrections for this diminution. For the purpose of estimating total base the following statistically derived regression formula, which corrects for protein, was shown best to fit our data:

$$\text{T.B.} = 10.19(\text{K} \times 10^3) - \frac{60.45}{\text{Pr}} + 35.16$$

where T.B. = total base mEq./L.; K $\times 10^3$ = conductance $\times 10^3$ at 25 C.; and Pr = Gm. protein per 100 ml.

This equation is in accord with the experimental observations of diminishing conductivity with increasing concentrations of protein. For making protein corrections, estimation of serum protein from measurement of the refractive index[5] has proved to be rapid, easy to perform, and economical of material, requiring only one drop of serum; no reagents are necessary, only the use of a refractometer being required.

When total base estimations are used as a rapid guide for replacement therapy, the measurement of serum protein is not always necessary. Under such conditions calculation can be made on the assumption of an average normal value for serum protein. For example, if the serum protein concentrations are within the normal range of values for bedfast individuals (*i.e.*, 6.0 to 6.9 Gm. per 100 ml.) an error of less than one per cent is introduced if a mean value of 6.38 Gm. per 100 ml. is assumed. If the serum proteins are decreased to 5 Gm. per 100

ml., an error of approximately only 2 per cent is introduced when a mean value is assumed. For many clinical purposes, therefore, the total base may be calculated with sufficient accuracy without making the additional measurement of protein.

When a mean value of serum proteins is assumed to be 6.38 Gm. per 100 ml., then the concentration of total base may be calculated by the formula:

$$\text{T.B.} \times 10.19\ (\text{K} \times 10^3) + 24.47$$

For convenience a nomogram[2] has been constructed with which the values for total base may be read directly either with or without protein measurement (Fig. 2).

CARE OF THE CELL

It may be regarded as an indication that the electrodes in the cell need replatinizing when they seem to be polarized, *i.e.*, when the bridge is balanced, spontaneous fluctuations occur in the opening of the "magic eye". The method for replatinizing the electrodes has been described previously.[6]

When serum is removed from the cell, the lumen should be immediately washed out with physiologic salt solution (0.9 per cent NaCl) and followed with distilled water. It is important to rinse the cell first with physiologic saline in order to prevent the precipitation of globulins. When measurements are made on more than one serum and sufficient amounts of serum are available, it is not necessary to dry the cell between measurements. Under such conditions, the cell is rinsed with the serum to be measured and the measurement is made after refilling.

The value for the cell constant should be checked periodically. We employ a sodium chloride solution of 140 milliequivalents (mEq.) per liter* as a convenient standard. This solution has a specific conductivity of 14.58×10^{-3} mhos at 25 C. The conductivity cell containing sodium chloride is placed in a water bath at 25 C.; the temperature compensator is then set at 25 C. and the main dial at 14.58 mhos $\times 10^{-3}$. The dial of the cell constant compensator is then adjusted until the "magic eye" indicates that the bridge is in balance. This reading represents the value of the cell constant.

ESTIMATION OF SERUM SODIUM

Total base denotes the summation of the cations Na, K, Ca and Mg, the mean value of which, under normal resting and fasting conditions, is approximately 146 mEq./L. $\pm$ 3. Of this total concentration, the mean summation value of K, Ca and Mg is 10.7 mEq./L. or approximately 7 per cent of the total base. This summation value is maintained relatively constant (*i.e.*, within $\pm$ 2 or 3 mEq.) in both normal and pathologic serums. For example, the mean value for potassium is 3.9 mEq./L. and values outside of the range of 3 and 6 mEq./L. are seldom observed even in pathologic conditions. Likewise, the mean value for serum calcium is 5 mEq./L. and values outside of the range of 3 to 7 mEq./L. are infrequent. The normal mean value for magnesium is 1.9 mEq./L. and in

*8.183 grams of fused sodium chloride dissolved in distilled water and diluted to 1 liter.

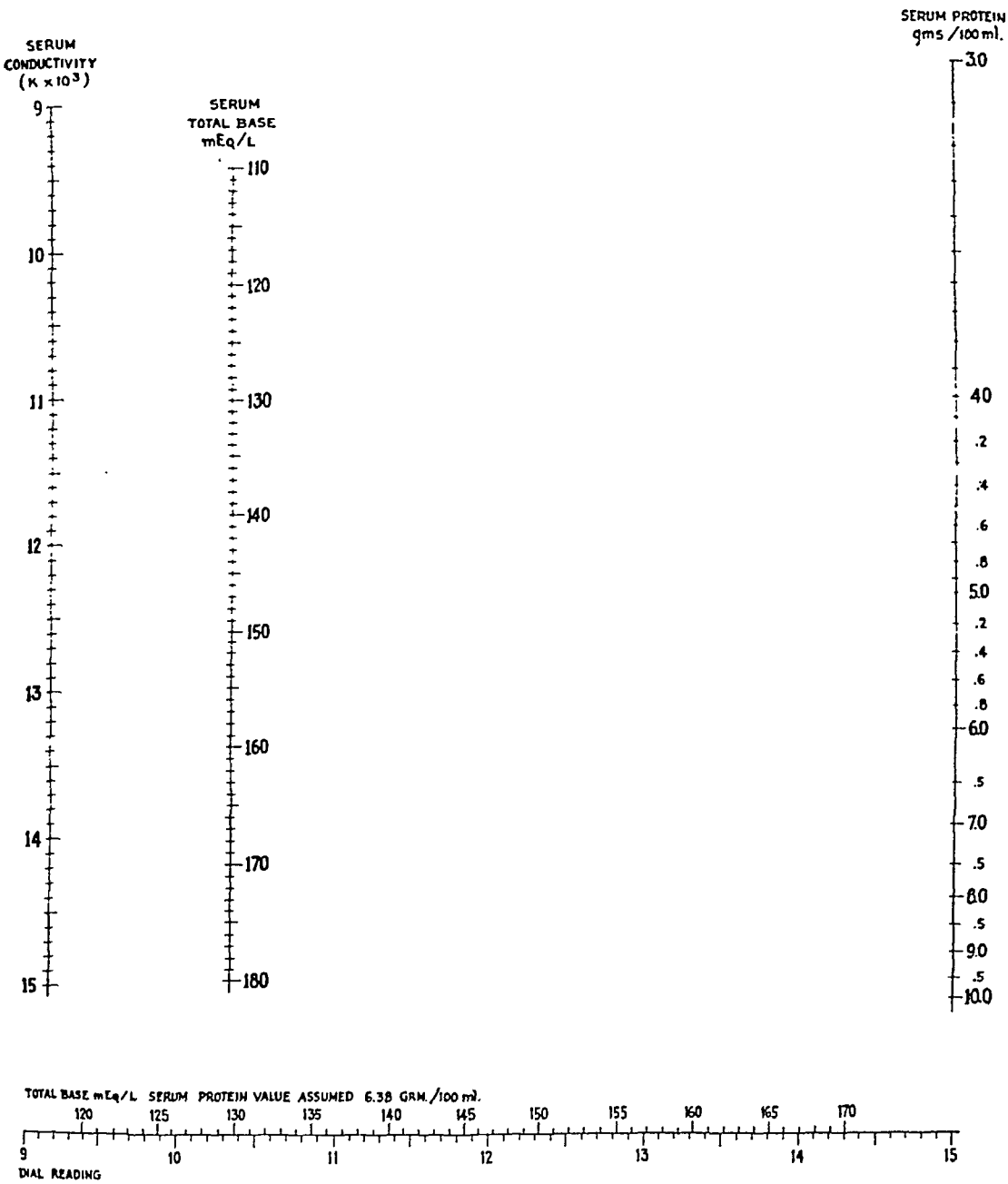


FIG. 2

pathologic conditions the deviations are practically always less than ± 1 mEq./L. Thus, owing to the relative constancy of the summation value of potassium,

calcium and magnesium, it is possible to make a rapid estimation of the sodium concentration for clinical purposes by subtracting 11 mEq. from the total base values.

SUMMARY

An improved design of conductivity bridge and pipet cell has been described for the rapid measurement of serum total base and the estimation of serum sodium.

REFERENCES

1. BUGARSKY, S., AND TANGL, F.: Physikalish chemische Untersuchungen über die molecularen Concentrationsverhältnisse des Blutserums. Arch. ges. Physiol. **72**: 531–565, 1898.
2. LUFKIN, H. M., AND SUNDERMAN, F. W.: Nomogram for calculating serum total base. Am. J. Clin. Path., **16**: 118–119, 1946.
3. SUNDERMAN, F. W.: A sixty cycle conductivity assembly for biological fluids. J. Biol. Chem., **88**: 61–66, 1930.
4. SUNDERMAN, F. W.: Studies in serum electrolytes; estimation of total base in serum. J. Biol. Chem., **143**: 185–190, 1942.
5. SUNDERMAN, F. W.: A rapid method for estimating serum proteins; formula for calculating serum protein concentration from the refractive index of serum. J. Biol. Chem., **153**: 139–142, 1944.
6. SUNDERMAN, F. W.: Measurement of serum total base. Am. J. Clin. Path., **15**: 219–222, 1945.

RAPID MACROSCOPIC TEST FOR INFECTIOUS MONONUCLEOSIS

A Method for Preservation of Sheep Cells*

F. RAPPAPORT, Ph.D., and M. SKARITON, M.Sc.

From the Bacteriologic Laboratory, Hadassah Hospital, Tel-Aviv, and the Biochemical Laboratory, Beilinson Hospital, Petah-Tikvah, Israel

The ordinary method of blood examination for heterophile antibodies is time-consuming and requires fresh sheep-blood. We present a rapid qualitative and quantitative method for the determination of heterophile antibodies and a technic for preservation of sheep's blood cells.

PROCEDURE

The preliminary qualitative method is a slide agglutination test. In most cases active serum is used. To one drop of the unknown serum (fresh or inactivated) add one loopful of concentrated sheep's blood corpuscles, washed 3 or 4 times with saline solution, and mix thoroughly with a glass rod. If no agglutination appears, the serum is considered to be negative. A typical agglutination constitutes a positive reaction and then requires quantitative evaluation.

According to the methods of Hattersley,[3] Evans[2] and Straus,[4] inactivation and incubation of serum is omitted. A series of dilutions is made, ranging from 1:7 to 1:3584 (see Davidsohn[1]).

Place 0.4 ml. of physiologic salt solution into the first tube and 0.25 ml. into each of the remaining 9 tubes. To the first tube, 0.1 ml. of the patient's serum is added and mixed well. From this tube 0.25 ml. is transferred to the second tube and mixed. From the second tube 0.25 ml. is withdrawn and transferred to the third, and so on. From the last tube, 0.25 ml. of the mixture is discarded. Then to each tube 0.1 ml. of the suspension of washed sheep-cells is added, and the contents are mixed by thorough shaking. The tubes are centrifuged immediately for two minutes at 3000 r.p.m. The results are read after gentle shaking.

The last tube containing single clumps indicates the limit of reaction. By using this method, it is not necessary to incubate or keep the material overnight in the ice box. In using preserved sheep-cells, serum dilutions have to be made with Borax-NaCl solution (Reagent 2) instead of the usual dilutions with 0.9 per cent NaCl.

In our experiments, every serum was tested by 3 methods: the qualitative slide method, the original Paul-Bunnell method (with inactivated serum) and the quantitative rapid method (Table 1). The results obtained by these methods were identical. The qualitative test first gives a positive result when the qualitative test shows agglutination in a dilution of 1:56. This limit should be re-

* Received for publication, March 14, 1949.

garded as suspicious for infectious mononucleosis, while a dilution of 1:112 constitutes a positive reaction.

We wish to emphasize the necessity of washing the sheep cells, as unwashed cells will give a negative reaction, even with strongly positive serums. In order to carry out the test rapidly, washed sheep-cells are kept on hand. Since sheep's blood keeps in the refrigerator for about five days only, it may be difficult in

TABLE 1

COMPARISON OF RESULTS USING FRESH AND PRESERVED SHEEP'S BLOOD CELLS WASHED WITH SALINE OR BORAX-SALINE SOLUTIONS

	DILUTIONS							
	1:7	1:14	1:28	1:56	1:112	1:224	1:448	1:896
Case 1 (normal)								
* Cells A	++	+	±	−	−	−	−	−
Cells B	++	+	±	−	−	−	−	−
Cells C	++	+	±	−	−	−	−	−
Case 2 (normal)								
Cells A	++	++	+	±	−	−	−	−
Cells B	++	++	+	−	−	−	−	−
Cells C	++	++	+	±	−	−	−	−
Infectious mononucleosis								
Cells A	++	++	++	++	++	+	−	−
Cells B	++	++	++	++	++	±	−	−
Cells C	++	++	++	++	++	±	−	−
Infectious mononucleosis								
Cells A	++	++	++	++	++	++	±	−
Cells B	++	++	++	++	++	++	−	−
Cells C	++	++	++	++	++	++	−	−
Diluted amboceptor 1:20 with negative human serum								
Cells A	++	++	++	++	++	++	−	−
Cells B	++	++	++	++	++	+	−	−
Cells C	++	++	++	++	++	+	−	−

* Cells A, fresh sheep-blood cells washed with 0.9 per cent NaCl.
Cells B, fresh sheep-blood cells washed with Borax-NaCl solution (Reagent 2).
Cells C, preserved sheep-blood cells washed with Borax-NaCl solution.

routine laboratory work to keep a supply of sheep's blood on hand that is satisfactory for use.

METHOD OF PRESERVATION OF SHEEP CELLS

We found that by adding an equal part of Borax solution (Reagent 1) of pH 7.4–7.6 to defibrinated sheep's blood, the latter can be preserved in the ice box for at least two months without undergoing hemolysis. The addition of Borax does not interfere with the reaction.

Reagents:

1. Reagent 1 (5 per cent Borax solution). 10 Gm. of crystallized Borax is

dissolved in 150 ml. of distilled water and concentrated citric acid solution added to obtain a pH of 7.4–7.6. Water is added to make a volume of 200 ml. The solution is then sterilized.

2. Reagent 2 (diluted Borax-sodium chloride solution). One part of Reagent 1 is mixed with 3 parts of 0.9 per cent saline solution. This solution may be stored for use.

Equal parts of the buffered Borax solution (Reagent 1) and defibrinated fresh sheep's blood are mixed. The mixture is placed into a number of test tubes (8 to 10) which are stored on ice. For use, the contents of one test tube are washed according to the following method:

To one part of the unwashed, stored sheep-blood cells poured into an Erlenmeyer flask, 3 parts of diluted Borax-saline solution (Reagent 2) are added, drop by drop, with gentle shaking. The resulting mixture is then poured into a test tube and centrifuged. The supernatant is removed by suction with a capillary pipet, and 3 to 4 volumes of Borax-saline solution is added. (The latter need not be added drop by drop.) Centrifugation and removal of the supernatant is repeated 2 more times.

The first washing must be done carefully, since during the long storage period the red blood corpuscles become accustomed to the highly hypertonic solution and on rapid contact with isotonic saline may be hemolyzed. After the third washing, the sediment of blood cells is used for qualitative and quantitative testing and then resuspended in Borax-saline and stored again in the refrigerator. This cell solution may be used until hemolysis is evident. It is advisable to use the contents of one tube for only one week.

SUMMARY

1. A qualitative macroscopic rapid slide method is described as a preliminary test for the presence of heterophile antibodies. In case this preliminary test is positive, a quantitative method is then used for determination of the titer.

2. These tests have the following advantages, as compared with the original methods: inactivation is omitted and results may be obtained quickly, since it is not necessary to incubate for 2 hours or store on ice for 24 hours.

3. A method for preserving sheep's blood cells is described.

REFERENCES

1. Davidsohn, I.: Serologic diagnosis of infectious mononucleosis. J. A. M. A., **108**: 289–295, 1937.
2. Evans, A. S.: Simplified "qualitative" method for heterophile antibody determination using capillary blood and white cell pipette. J. Lab. and Clin. Med., **32**: 1278–1281, 1947.
3. Hattersley, P. G.: New rapid technique for Rh typing. J. Lab. and Clin. Med., **32**: 1024–1026, 1947.
4. Straus, R.: Simple slide and tube tests for infectious mononucleosis. Am. J. Clin Path., **6**: 546–556, 1936.

EXCRETION OF SILVER IN A PATIENT WITH ARGYROSIS

Ineffectiveness of Treatment with BAL*

FRANCIS S. GERBASI, M.D., and ABNER R. ROBINSON, M.S.

From the Department of Surgery of The Alexander Blain Hospital and The Research Laboratory of the Children's Fund of Michigan, Detroit, Michigan

An accurate spectrometric analysis in a patient suffering from chronic argyrosis was recently undertaken to determine the effect of British Anti-Lewisite (BAL) on the course of the disease. To our knowledge, no similar method of study has been reported on the effect of BAL in human argyrosis.

Qualitative studies in rats have suggested that BAL (2,3 dimercaptopropanol) is ineffective in the treatment of chronic silver poisoning.[16] It has been used clinically and experimentally in the treatment of many of the diseases produced by heavy metal and has been found to be effective in intoxication by arsenic,[6] mercury,[11] gold,[10, 12] antimony,[3] bismuth,[3] chromium,[3] nickel,[3] calcium,[8] zinc,[1, 13] and copper.[14]

CLINICAL HISTORY

J. J., Case No. H-775, A.B.H., a 60 year old man, was first seen at the hospital on March 19, 1947, complaining of discoloration of his eyes since 1923. He was a tool and dye maker, who in 1917, sustained a foreign body (emery) injury to his eye. A physician removed the foreign body and prescribed 10 per cent Argyrol (a silver protein preparation) eye drops twice daily. The patient continued to instill these drops off and on for seven years, whenever he felt the slightest irritation of the eyes.

In 1917, during the influenza epidemic, he used 5 per cent or 10 per cent Argyrol solution almost constantly both as nose drops and as gargles, in the belief that it would prevent him from contracting the disease.

In 1923, he first noticed a grayish discoloration of his eyes. He then discontinued the use of the Argyrol eye drops, but persisted in the use of the silver preparations as nose drops.

In 1927, upon the advice of a druggist, the patient started to use Neo-silvol (silver nucleinate) freely instead of Argyrol, "since this was white and would not cause any discoloration". In 1930 he first noticed a grayish discoloration of his skin and, therefore, stopped using all silver preparations. The patient had never been treated for argyrosis. At the present time he believes his skin is lighter than it was in 1930.

On examination on March 19, 1947 the patient was well developed and well nourished, appeared to be about 50 years of age and was active and alert. His hands and feet had an ashen blue color, while the covered surfaces of his body had a lighter grayish color. The conjunctivae and sclerae were noticeably blue. No blue lines were visible on his gums, but his nasal and buccal mucous membranes were light bluish gray. The rest of the physical examination was essentially negative. Urinalysis and complete blood count were negative. Urinary excretion of phenolsulphonphthalein in two hours was 75 per cent; fasting blood sugar, 86 mg. per 100 ml. An electrocardiogram showed no evidence of organic heart disease and an x-ray film of the chest revealed mild tortuosity of the aorta.

METHOD

Twenty-four hour specimens of urine and feces were collected prior to treatment. On April 21 and 22, 1947 he was given four injections daily, each of 2

* Received for publication, April 8, 1949.

ml. of BAL (2.5 mg. per kilogram) intramuscularly. A 10 per cent solution of BAL in peanut oil with 20 per cent benzyl benzoate was used. From April 23 to April 29, inclusive, he received one injection daily of 2 ml. of BAL. During the period of treatment all specimens of his urine and feces were saved and spectrometrically analyzed. A piece of skin was removed for biopsy before and after treatment.

FINDINGS

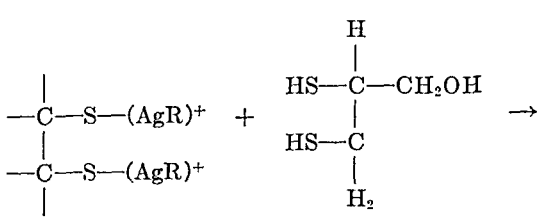
The samples of urine were analyzed by the Lundegardh flame technic[4] and the copper spark technic.[7] With the flame technic for silver the urine samples contained less than 5 parts per million (Fig. 2). The copper spark method, which is more sensitive but less accurate, determined the amount of silver present to be 8×10^{-8} Gm. per ml., and no significant difference was found in the urinary values before and after treatment (Fig. 3).

The fecal samples were ashed and analyzed by the copper spark method. The fecal samples varied considerably in silver content but the highest value was obtained in the sample on the day before instituting treatment with BAL. The values varied from 3×10^{-6} Gm. to 3×10^{-7} Gm. per day (Fig. 4). No determinations were made of the amount of silver ingested in the diet.

The skin samples from the patient were analyzed before and after treatment by the copper spark technic and found to contain 4.0×10^{-6} Gm. of silver per Gm. of dry skin before treatment and 3.7×10^{-6} Gm. of silver per Gm. of dry skin after. The difference in the two determinations was well within the limits of error of the method (Fig. 5). Microscopic examination of the skin revealed deposition of silver in the upper layers of the corium, beneath the epithelial layer, and around sweat and sebaceous glands (Fig. 1).

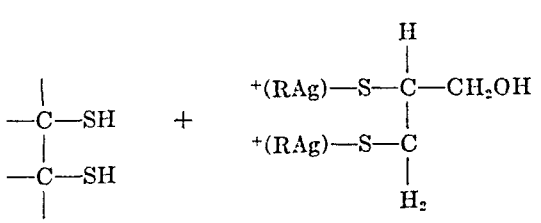
DISCUSSION

The exact compound of the silver deposited in the tissues is not known. The silver might be combined with the sulfhydryl groups of the tissue proteins in a



TISSUE THIOL SILVER (not stable)

BAL



FREE TISSUE THIOL

BAL-THIOL SILVER (stable)



FIG. 1. A. Hematoxylin and eosin stain of section of skin showing faintly black particles of silver deposit around the basement membrane of a sweat gland. × 950.

B. Dark-field examination of the skin, taken from the same block of tissue as A, from the argyrial patient, showing the deposition of silver (white dots) around a sweat gland. × 950.

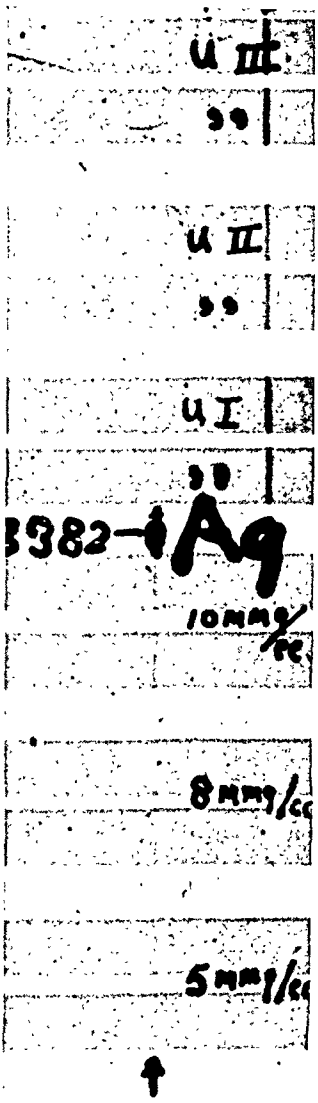
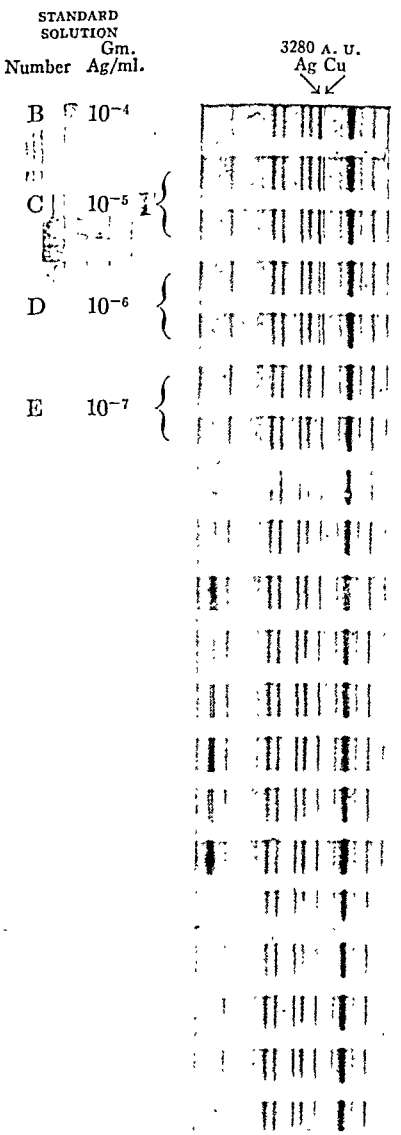


FIG. 2. Spectrograms obtained by the Lundegardh flame technic. In the spectrograms of the lower half of the figure, the arrow points to faint lines obtained from standard solutions of silver (5, 8 and 10 micrograms per ml. solution). No comparable lines are present in the upper three spectrograms of urine of the patient, indicating less than 5 parts per million of silver in the urine. The solid lines at the right were produced by cobalt standards. U I represents urine specimen before treatment with BAL, U II and U III specimens of urine obtained during treatment.

manner similar to arsenic, in which case BAL, with its two dithiol groups, could compete with these tissue thiol-silver compounds and, thus, make available a stable BAL-thiol silver compound which could be readily excreted.

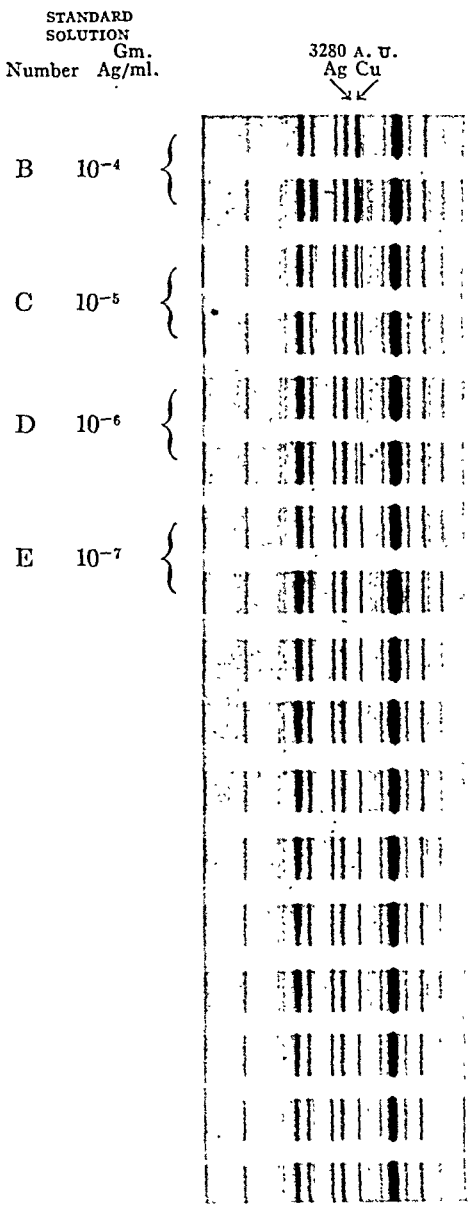


URINE SPECIMEN	DAY OF TREATMENT	DAILY VOLUME URINE *ml.*	Ag CONTENT *Gm. per 100 ml.*
U I	7 days before	850	9×10^{-6}
U II	1 day after	980	8×10^{-6}
U III	2 days after	750	7×10^{-6}
U IV	4 days after	1160	9×10^{-6}
U V	5 days after	1240	1×10^{-5}
U VI	7 days after	890	7×10^{-6}
U VII	8 days after	950	8×10^{-6}
U VIII	10 days after	1700	9×10^{-6}
Cu blank			

FIG. 3. Spectrograms of specimens of urine obtained before and after onset of treatment with BAL. The silver lines are readily visible in the standard solutions, the relative density being proportional to the quantity of silver present in the specimen. In the urine specimens the silver lines are barely perceptible and the differences in silver content are seen to be insignificant (.00001–.000009 Gm./100 ml. of urine). A standard copper line was photographed in juxtaposition to facilitate the location of the silver line.

This theory has been supported by reports of improvement in the color of the skin of argyrial patients, following the injection of sodium and calcium thiosulphate.[5] Other investigators,[17, 18] however, have not been able to confirm

this. Our results also do not support this theory, since there were no apparent changes in the amount of silver in the skin, urine or feces after treatment.

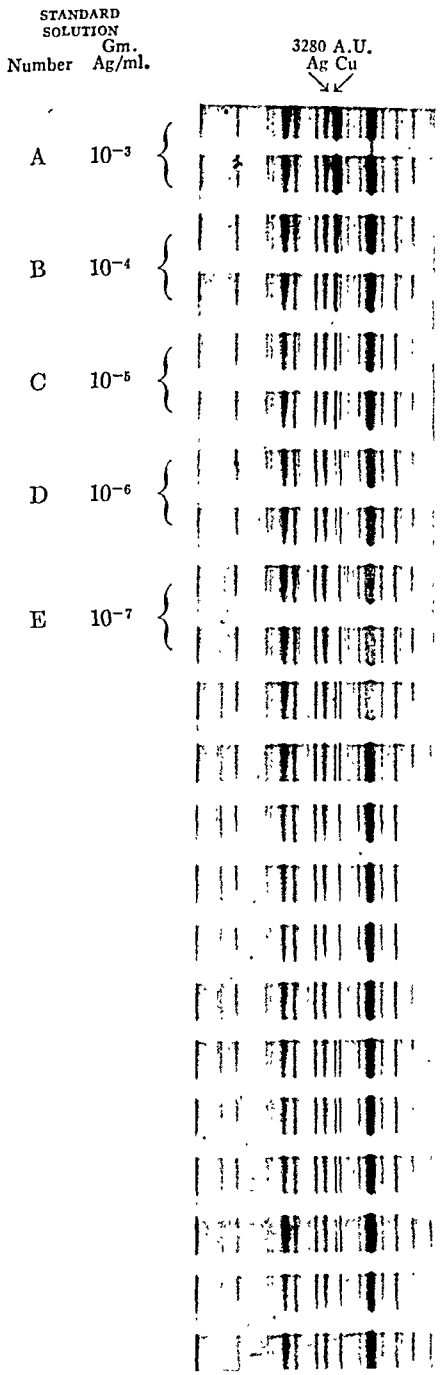


SPECIMEN	DAY OF TREATMENT	GM. Ag IN FECES Per Day
I	8 days before	3×10^{-6}
II	7 days before	2.4×10^{-6}
III	2 days after	1.0×10^{-6}
IV	5 days after	2.0×10^{-6}
V	7 days after	3.0×10^{-7}
Cu blank		

FIG. 4. Spectrograms of fecal specimens taken before and after beginning of treatment with BAL. Here as in the spectrograms of the urine (Fig. 2) the silver lines are readily visible in the standard solutions but almost imperceptible in the fecal specimens. The amount of silver in the fecal specimens is variable and small but the highest amount was in the sample taken before treatment was begun (.000003 Gm./day).

Contrary to previous reports,[2] however, a very small amount of silver was present in the urine and feces of our patient with chronic argyria universalis.

Although we found BAL to be ineffective in our patient with chronic argyrosis, this agent may still be of value in acute silver poisoning. If it is true that the



SKIN TISSUE TESTED FOR SILVER		
Source	Date Excised	Gm. Ag/Gm. Dry Skin
Patient	April 21, 1947	4.0×10^{-6}
Normal		None
Normal		None
Patient	July 6, 1948	3.7×10^{-6}
Patient	July 6, 1948	3.7×10^{-6}
Cu blank		

FIG. 5. Spectrograms of normal skin and skin taken from patient with argyrosis. BAL was administered from April 21, 1947 to April 29, 1947. The silver lines are readily visible in the spectrograms of the skin of the argyrial patient but are not visible in those from the normal skin. The amount of silver in the skin before and after treatment is practically the same (.0000036 and .000004 Gm./Gm. of dry skin).

silver is at first loosely combined with the sulfhydryl groups of the tissue proteins and is later reduced to an insoluble silver compound, it is possible that if BAL is given early enough, it may be able to mobilize the silver and thereby prevent the development of argyrosis.

Another factor to be considered in attempting to explain the failure of BAL in the excretion of silver, is the extremely low coefficient of solubility of silver (Table 1). Neuberg,[15] however, is of the opinion that the coefficient of solubility of the heavy metals is of very little importance and suggests that amino acids, particularly alpha and beta amino acids, beta alanine, and related compounds are important in holding relatively insoluble heavy metals in solution or dissolving them.

TABLE 1

COEFFICIENTS OF SOLUBILITY OF CHLORIDES OF HEAVY METALS IN WATER*

SALT	SOLUBILITY IN GM. PER 100 ML. OF WATER AT 25 C.
Silver chloride	0.000089
Copper chloride	70.6
Lead chloride	0.673
Manganese chloride	62.2
Mercurous chloride	0.00021
Mercuric chloride	6.9
Arsenic chloride	(Decomposes)
Gold chloride	68.0
Bismuth chloride	(Decomposes)
Chromium chloride	(Very soluble)
Nickel chloride	64.2
Calcium chloride	59.5
Zinc chloride	262.0

* Lange, N. A.: Handbook of Chemistry. Ed. 6. Sandusky, Ohio: Handbook Publishers Inc., 1946.

Klotz,[9] who treated a patient with argyrosis with BAL without effect, used chemical and not spectrometric methods of analysis.

SUMMARY

In a patient with chronic argyrosis who was treated with BAL (2,3 dimercaptopropanol), quantitative studies of the amounts of silver in the urine, feces and skin were made by means of the Lundegardh flame or the copper spark technic.

Although small amounts of silver were excreted in the urine of this patient, no appreciable differences were detected in the excretion of silver either in the urine or in the feces before and after treatment, nor was there any change in the concentration of silver in the skin before and after treatment.

REFERENCES

1. American Work on BAL: Editorial. Nature, London, 157: 218, 1946.
2. BLUMBERG, H., AND CAREY, T. N.: Argyremia. J. A. M. A., 103: 1521–1524, 1934.

3. Braun, H. A., Lusky, L. M., and Calvery, H. O.: The efficacy of 2,3-dimercaptopropanol (BAL) in the therapy of poisoning by compounds of antimony, bismuth, chromium, mercury and nickel. J. Pharmacol. and Exper. Therap., (Suppl.) 87: 119–125, 1946.
4. Briggs, Mary A., Johnston, Ruth, and Elledge, Bonnie E.: Mineral analysis of biological materials. Indust. and Engin. Chem., Anal. Ed., 13: 99–101, 1941.
5. Cannon, A. B.: Value of silver arsphenamine in the treatment of early syphilis; conclusions based on study of 104 cases. J. A. M. A., 102: 268–273, 1934.
6. Eagle, H.: The systemic treatment of arsenic poisoning with BAL (2,3-dimercaptopropanol). J. Ven. Dis. Inform., 27: 114–121, 1946.
7. Fred, M., Nachtrieb, N. H., and Tomkins, Frank S.: Spectrochemical analysis by the copper spark method. J. Optic. Soc. America, 37: 279–288, 1947.
8. Gilman, A., Allen, R. P., Phillips, F. S., and St. John, E.: Clinical uses of 2,3-dimercaptopropanol (BAL); the treatment of acute systemic mercury [illegible] mental animals with BAL thiosorbitol and BAL glucoside. J. [illegible] 25: 549–556, 1946.
9. Klotz, Arthur P.: Personal communication to the authors.
10. Lockie, L. M., Norcross, B. M., and George, C. W.: Treatment of two reactors due to gold; response of thrombopenic purpura and granulocytopenia to BAL therapy. J. A. M. A., 133: 754–755, 1947.
11. Longcope, W. T., and Leutscher, J. A., Jr.: Clinical uses of 2,3-dimercaptopropanol (BAL); the treatment of acute mercury poisoning with BAL. J. Clin. Investigation, 25: 557–567, 1946.
12. Margolis, H. M., and Caplan, P. S.: BAL in the treatment of toxicity from gold. Ann. Int. Med., 27: 353–360, 1947.
13. McCance, R. A., and Widdowson, E. M.: Observations on the administration of BAL -Intrav to man. Nature, London, 157: 837, 1946.
14. McDonald, I. W.: Effect of BAL-Intrav on excretion of copper by the sheep. Nature, London, 157: 837, 1946.
15. Neuberg, Carl, and Mandl, Ines: Unknown effect of amino acids. Arch. Biochem., 19: 149–161, 1948.
16. Olcott, Charles T., and Riker, Walter F., Jr.: Experimental argyrosis; treatment of rats receiving silver with 2,3-dimercaptopropanol (BAL). Science, 105: 67, 1947.
17. Stillians, A. W.: Argyria. Arch. Dermat. and Syph., 35: 67–77, 1937.
18. Wollenberg, R. A.: Argyria. Arch. Dermat. and Syph., 16: 242, 1927.

PHOTOGRAPHIC RECORDING OF HEMOLYSIS USING A PORTABLE ELECTROCARDIOGRAPH*

WARNER E. LOVE, B.A., ROBERT EDELBERG, B.S., AND EDWARD F. MACNICHOL, A.B.

From The Department of Physiology and The Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia, Pennsylvania

The apparatus for obtaining photographic records of hemolysis, devised by Parpart[3] and used with modifications by Jacobs, Stewart, Brown and Kimmelman[2] in their standard method for the detection of osmotic abnormalities of erythrocytes, is somewhat difficult to use and is not generally available. In an attempt to provide a method more useful for clinical purposes, the Sanborn "Cardiette", a portable electrocardiograph, has been suitably modified to yield records which in some respects are superior to those obtained with the original apparatus. The modified instrument has been found to be reliable and to give reproducible records. More than 2000 curves have been recorded with the present apparatus; some of them form the basis for two recent papers dealing with clinical problems.[1, 4]

The Sanborn instrument, as it stands, is unsuitable for recording hemolysis in three respects: (a) The speed of movement of the photographic paper is much too great; (b) any considerable slowing is impossible without excessive fogging of the white tracing on its black background; and (c) the instrument is incapable of maintaining a given deflection for more than about 0.5 second and, therefore, cannot be used to record constant or slowly-changing conditions. The simple and readily reversible modifications necessary to remedy these defects will be described in order.

MODIFICATIONS OF THE SANBORN INSTRUMENT

1. Slowing of the paper is accomplished by connecting the shaft of the gear which carries the stroboscopic disc on the back of the electrocardiograph with a slow synchronous motor,† thereby retarding the spring-driven mechanism. With this arrangement a three minute record requires only 3 cm. of paper.

2. With such a slow speed, the usual white tracing on a black background would be fogged by stray light. This difficulty is avoided by obtaining a black tracing on a white background. The usual white tracing is formed by the shadow of a post in the path of a beam of light incident on the galvanometer mirror. The desired reversal is effected by substituting for the post a piece of sheet metal with a vertical slit. The post is located under the panel of the electrocardiograph, between the light source and the galvanometer; it is exposed by removing the batteries.

3. The inability of the circuit to record slow changes is overcome by interrup-

* Received for publication, April 15, 1949.

† Synchron, Model 600, 2 r.p.m., Hansen Mfg. Co. Inc., Princeton, Indiana.

tion of the input potentials. This is done by flashing light through the hemolyzing suspension at fixed intervals. A photovoltaic cell picks up these flashes and converts them to electrical impulses which are then recorded photographically by the electrocardiograph.

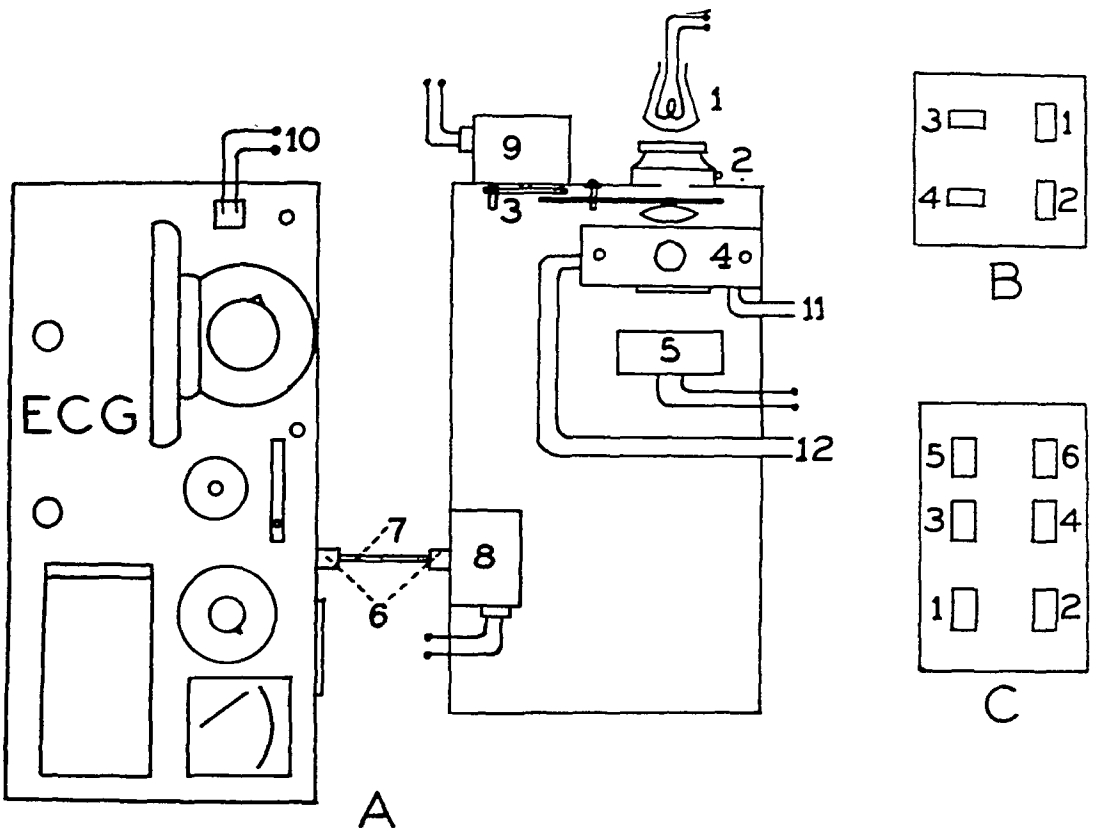


FIG. 1. A. Diagram of auxiliary unit showing coupling with electrocardiograph: (1) 6 volt automobile head-lamp; (2) diaphragm; (3) light-interrupting mechanism; (4) water bath; (5) photocell; (6) rubber pressure tubing; (7) dowel; (8) 2 r.p.m. synchronous motor; (9) 10 r.p.m. synchronous motor; (10) patient-leads to photocell; (11) water inlet: (12) water outlet.

B. Patient lead-cable receptacle: (1) negative lead from photocell; (2) open; (3) positive lead from photocell; (4) ground.

C. External battery receptacle on electrocardiograph: (1) −1.5 v.; (2) −45 v., +1.5 v.; (3) +22.5 v.; (4) open; (5) +45 v.; (6) ground.

THE PHOTOELECTRIC SYSTEM

The auxiliary unit is a light-tight box (Fig. 1A) which contains the photoelectric system and the motor for slowing the camera. The photoelectric system consists of (1) a light source, (2) a diaphragm, (3) a light-interrupter, (4) a water bath and (5) a photocell and cable. These will be described in order:

1. The light source is a 50 C.P. automobile head-lamp bulb powered by a heavy-duty, 6 volt, 150 amp. hr. storage battery.

2. To regulate the amount of light in the photoelectric system a diaphragm from a microscope substage is placed between the light source and the light-interrupter.

3. The light-interrupter is a lever-operated shutter, activated by a cam on the shaft of a 10 r.p.m. synchronous motor.* It regulates the timing of the flashes of light so that a 0.5 second flash occurs every 6 seconds. The 5.5 second interval between the flashes allows the galvanometer to return to its rest position.

4. The water bath surrounds the test tube containing the suspension. Masks are so arranged that light leaving the bath has passed through the suspension but not around it. A 5 cm. lens, on the illuminated side of the water bath, acts as a condenser, and a piece of ground glass on the other side diffuses the transmitted light. To insure temperature regulation, water is siphoned into the bath from a thermostatically-controlled reservoir. An outflow of larger diameter placed above the optical path insures a constant water level.

5. A photovoltaic cell, Weston Model 594 RR, is centered approximately 3 cm. behind the ground glass. Figure 1B shows the wiring of the patient-lead receptacle on the panel of the electrocardiograph, when "lead I" is used. The photocell is connected to the electrocardiograph through this receptacle by a suitable plug. The lead-cable and the photocell socket must be shielded and the shielding grounded to the chassis of the electrocardiograph.

RECORDING HEMOLYSIS

A base line of zero per cent hemolysis is first established. A depressed signal point on the record indicates zero time on the hemolysis curve which follows; another signal point separates the end of the experimental curve from a tracing, indicating the position of 100 per cent hemolysis. Figure 2 shows several typical records.

A final volume of solution of 5 ml. is recommended. Four pyrex 15 x 150 mm. rimless test tubes (numbered 1 to 4) and one pyrex 16 x 100 mm. rimmed test tube (numbered 5) are used. Tube 1, representing zero per cent hemolysis, contains 1 ml. of standard suspension and 4 ml. of 0.9 per cent NaCl. Tube 2 contains 4 ml. of hemolytic agent and is the tube in which hemolysis occurs. Tube 3, representing 100 per cent hemolysis contains 2 ml. of standard suspension and 8 ml. of water plus 2 drops of a concentrated saponin solution. Tube 4 is a signal tube, not necessary for the experiment, but convenient. It contains a solution of hemoglobin, the optical density of which is such that it gives the second signal point. Tube 5 contains 1 ml. of the standard suspension which is mixed with 2 at the proper moment.

A base line is recorded by placing tube 1 in the instrument for a minute. The depressed signal point is made by introducing an opaque object into tube 1. At the instant the shutter opens to record this point, the contents of tube 2 are poured into tube 5 and the mixture is poured back into tube 2, which is then exchanged for tube 1. Hemolysis now occurs and is recorded. At the end of hemolysis or at some preselected time, tube 4 is exchanged for tube 2 and the second signal point is recorded. Finally, the contents of tube 2 are discarded

* Synchron, Model 600, 10 r.p.m.

and it is refilled with the contents of tube 3. The refilled tube is then exchanged for tube 4 and 100 per cent hemolysis is recorded. The use of the same tube for recording the optical density of the two different solutions obviates the necessity for matched tubes.

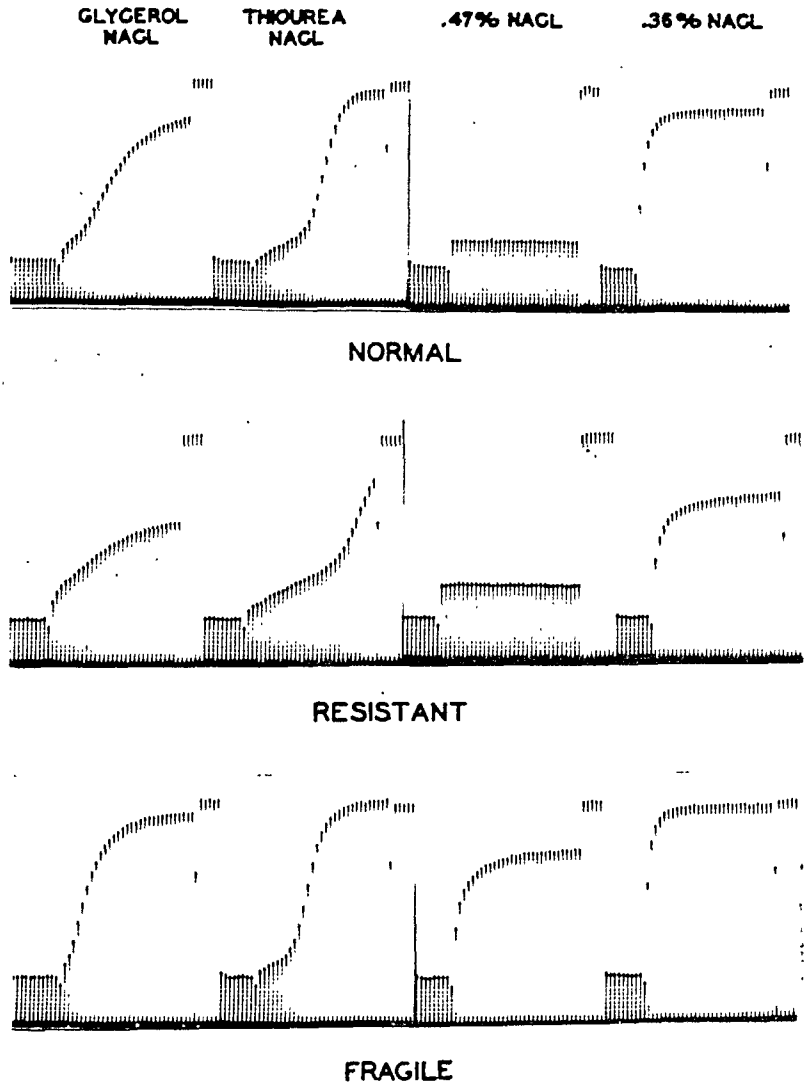


FIG. 2. Records of behavior of normal and abnormal bloods.

The timing blades that make the vertical lines on the usual electrocardiogram interrupt the light incident on the galvanometer every six seconds. In starting the motors, care must be taken that a blade is not obstructing the optical path to the photographic paper at the time the shutter in the photoelectric system is open. This is done by observing the beam in the window on the panel of the electrocardiograph.

External "A" and "B" batteries, as recommended by the Sanborn Co., should be used if the instrument is to be run for hours at a time. With a small resistance in series, one of the 2-volt cells in the heavy duty storage battery used to operate the light source may be used in place of the 1.5 volt "A" battery. The wiring of the external battery receptacle on the back of the electrocardiogram is shown in Figure 1C.

SUMMARY

A method of adapting a portable Sanborn "Cardiette" for recording hemolysis is described and sample records obtained with it are presented.

Acknowledgment. The authors are indebted to Professor M. H. Jacobs for his valuable advice, helpful suggestions, and encouragement during this work. They also wish to thank Professor L. Irving of Swarthmore College for the use of his electrocardiograph.

REFERENCES

1. DICKSTEIN, B., LANDMESSER, W. E., LOVE, W. E., WILSON, T. H., AND WOLMAN, I. J.: The osmotic resistance of human erythrocytes in normal, carrier, and anemic states; with special reference to changes due to age, race, sickle cell anemia, Mediterranean (Cooley's) anemia and congenital hemolytic icterus. Am. J. M. Sc., **217**: 53–61, 1949.
2. JACOBS, M. H., STEWART, D. R., BROWN, W. J., AND KIMMELMAN, L. J.: An improved method for the detection of osmotic abnormalities of erythrocytes. Am. J. M. Sc., **217**: 47–52, 1949.
3. PARPART, A. K.: The permeability of mammalian erythrocyte to deuterium oxide (heavy water). J. Cell. and Comp. Physiol., **7**: 153–162, 1935.
4. WILSON, T. H., AND LANDMESSER, W. E.: Osmotic resistance of erythrocytes from patients with carcinoma. Cancer, in press.

TEXCHROME SILHOUETTE STAINING OF PLATELETS

MORPHOLOGY *IN VITRO* BEFORE AND DURING COAGULATION*

L. VROMAN, M.S.

From the Research Division of the Laboratory, St. Peter's General Hospital, New Brunswick, New Jersey

The morphologic alterations in blood platelets *in vitro*, including formation of various types of surface projection and their behavior in the presence of fibrin have been described by Schimmelbusch,[8] Fonio and Schwendener,[4] Ferguson,[3] Best [1] and others. Darkfield microscopy has been extensively utilized in studying the progress of these changes, especially during clotting. By this means the migration of platelets toward fibrin needles and the process of clot retraction have been studied by Tocantins[10] in slowly-clotting hemophilic blood. More recently, the use of silicones[5] and various types of plastic coating to retard clotting *in vitro*, and observations by phase microscopy[6] have supplemented the older procedures.

In the course of our studies on the behavior of blood platelets, particularly in connection with morphologic alterations *in vitro* in relation to variations in platelet adhesiveness,[7] we have found Texchrome staining of advantage in making quick permanent stained mounts of platelets in clotting blood, studied successively in serial preparations. It has proved a most satisfactory stain for the demonstration of fibrin filaments in clotting blood as well as in fixed tissues. Through the kindness of Dr. H. L. Davis[2] of Johnson & Johnson Research Laboratories, a generous supply of Texchrome† was made available to us for this purpose.

METHODS

Fixation. A small drop of freshly drawn blood (or recalcified citrated blood) is placed on a clean slide. The latter is immediately covered with the top half of a petri dish, upon the under inner surface of which a moist disc of filter paper is affixed to retard drying. The specimen is permitted to stand at room temperature until the desired phase of clotting or clot retraction is achieved, at which time the petri-dish cover is removed and the slide gently rinsed with a few cubic centimeters of physiologic saline, in such a manner that the entire clot is washed off except for its more adherent narrow outer margin. An applicator may be used to assist in its removal. The wet slide is then flooded with absolute methanol for fixation. After three minutes or more the slide is washed with tap water.

Staining. While still wet the preparation is covered with Texchrome. After

* Received for publication, February 4, 1949.

† Texchrome is composed of acid fuchsin (color index No. 692), 6 Gm.; picric acid, 10 Gm.; tannic acid, 10 Gm.; National Soluble Blue 2 B Extra (color index No. 707), 5 Gm. The dyes may be ground together, and dissolved together, or separately in any order, and diluted to 1 liter. Texchrome is distributed by Eimer and Amend, New York, New York.

three or more minutes the slide is washed for a few seconds in tap water, dried in air and mounted with a cover slip.

COMMENT

This method of staining is not designed to supplant the use of Giemsa's stain, Mallory's phosphotungstic acid hematoxylin stain or other stains for fine histologic detail. The inner structure of the platelets is obscured in Texchrome preparations, possibly because of a delicate coating of plasma or fibrin, so that only the dark blue silhouette of the platelet appears. The cytoplasm of leu-

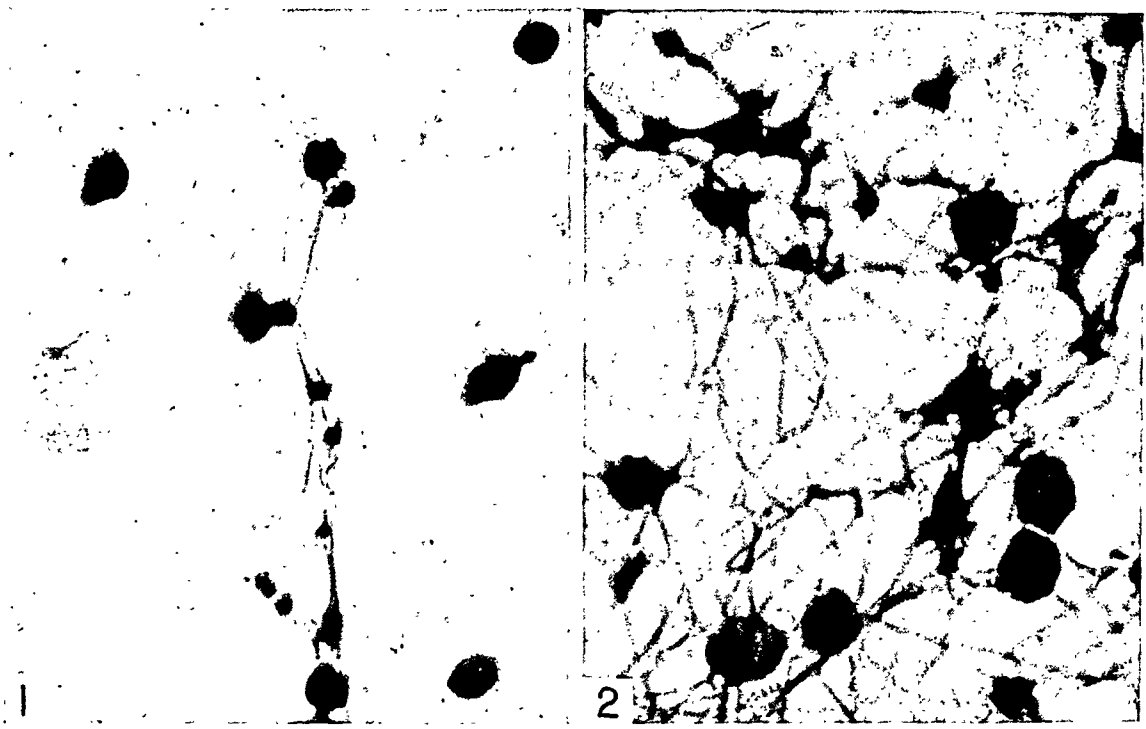


FIG 1. Photomicrograph of platelets undergoing lysis, showing marginal hyaline swelling of platelets, also strands of early fibrin. Texchrome staining. × 1000.
FIG. 2. Photomicrograph of fibrin clot, showing intact platelets forming orientation nodes of fibrin filaments with entrapped erythrocytes. Texchrome staining. × 1300.

kocytes stains pale blue, their nuclei darker blue. Erythrocytes stain yellow, brown, red or blue. When thin areas of normally adherent blood clot are examined, the fibrin filaments appear collected into well-defined threads, crisscrossing in the form of a web. The platelets give the appearance of having participated actively in the formation of the web by serving as centers or nodes of attachment of its component filaments. In preparations of normal blood, the majority of the platelets resemble spiders in the centers of radiating interlocking networks of fibrin threads while the remaining platelets, along with the red blood cells and leukocytes, appear to be caught passively like flies in a web. The morphology of platelets varies with duration *in vitro*, particularly with successive stages of clotting, as described below.

(a) *Fixation and staining before clotting.* Many platelets are found adherent to the glass slide, some being intact, others showing various types of surface projection (spines, knobs, vesicles) or fragmentation into large balloon-like or ovoid granules. Some of the platelets exhibit uniform swelling of their entire outer portion in the manner of an ectoplasm (Fig. 1).

(b) *Fixation and staining during clotting.* Many platelets are characteristically surrounded by balloon-like structures as a result of previous fragmentation, and are unencumbered with fibrin. Other platelets are still more or less intact and are entangled in a net of fibrin or adherent to loose fibrin threads, as described above (Fig. 2).

(c) *Fixation and staining during clot retraction.* In areas from which the clot has retracted, nearly all the platelets appear in various stages of fragmentation ("exploding platelets"), rarely with adherent fibrin.

When blood is placed on lucite slides or on slides coated with transparent paraffin or with silicone, platelet alterations and lysis and the clotting and retraction of fibrin are retarded or suppressed.

The above observations on clot retraction have been made repeatedly and suggest, among other things, that in the process of clot retraction the portion of clot attached to the glass surface of the containing vessel, through its anchorage of adherent platelets, is eventually separated from the glass by a type of platelet lysis, which, in turn, releases the fibrin. It may well be the case that the rate at which such lysis occurs determines the *clot retraction time* perhaps to an even greater degree than elastic forces within the substance of the clot produced by the twisting and bending of the threads of fibrin under the influence of intact platelets.[9]

In view of the important role of contact with a wettable surface in initiating platelet lysis *in vitro*, it is not difficult to interpret on this basis the findings by Tocantins,[10] that in platelet-rich plasma (in contrast to platelet-free plasma) the rate of clot retraction depends primarily upon the relative amount of glass surface to which clotting blood is exposed.

SUMMARY

Sharply defined silhouette staining of blood platelets and fibrin is accomplished easily with Texchrome, a polyvalent tissue stain. Many details of platelet changes following contact with glass and other surfaces, and in relation to clotting and clot retraction can be observed. Data thus obtained suggest that the rate of retraction of clot from glass surfaces depends to a larger extent upon lysis of the platelets which bind fibrin filaments to the glass surface than upon elastic forces developed within the clot itself.

Acknowledgment. The author wishes to thank Dr. Sylvan E. Moolten, Director of the Laboratory of St. Peter's Hospital, for his advice and assistance.

REFERENCES

1. Best, C. H., Cowan, C., and Maclean, D. L.: Heparin and the formation of white thrombi. J. Physiol., 92: 20–31, 1938.

2. DAVIS, H. L., AND RYNKIEWICZ, H. J.: A fiber identification stain. Indust. and Engin. Chem., Anal. Ed., **14:** 472, 1942.
3. FERGUSON, J. H.: Observations on the alterations of blood platelets as a factor in coagulation of the blood. Am. J. Physiol., **108:** 670–682, 1934.
4. FONIO, A., AND SCHWENDENER, J.: Die Thrombozyten des menschlichen Blutes und ihre Beziehung zum Gerinnungs- und Thrombosevorgang. Bern: Medizinischer Verl. Hans Huber, 130 pp., 1942.
5. JAQUES, L. B., FIDLAR, E., FELDSTED, E. T., AND MACDONALD, A. G.: Silicones and blood coagulation. Canad. M. A. J., **55:** 26–31, 1946.
6. MANN, F. D., HURN, M., AND MATHIESON, D. R.: Platelets as foci in the coagulation of blood. Federation Proc., **7:** 77–78, 1948.
7. MOOLTEN, S. E., AND VROMAN, L.: Glass-wool filter test for platelet adhesiveness. To be published.
8. SCHIMMELBUSCH, C.: Die Blutplättchen und die Blutgerinnung. Virchows Arch f. path. Anat., **101:** 201–244, 1885.
9. TOCANTINS, L. M.: Platelets and the spontaneous syneresis of blood clots. Am. J. Physiol., **110:** 278–286, 1934.
10. TOCANTINS, L. M.: Platelets and the structure and physical properties of blood clots. Am. J. Physiol., **114:** 709–715, 1936.

CULTURE OF TUBERCLE BACILLI FROM SPECIMENS OF GASTRIC JUICE*

PAUL KABLER, M.D., AND RUTH LUNDHOLM, B.S.

From the Section of Medical Laboratories, Minnesota Department of Health, University Campus, Minneapolis, Minnesota

It is known that some specimens of gastric juice have a bactericidal action on tubercle bacilli. The mechanism of this action has not been fully explained, although the effects of saliva[3, 7, 11] bile,[4] acid,[2] pepsin and a germicidal factor[1] in gastric juice have been studied. Gastric juices,[6, 8–10] both natural and artificial, have exhibited this inhibitory action, which seems to vary in intensity with the number of bacilli present, exposure time, and temperature, as well as with the inherent qualities of the juice itself.

The elapsed time from collection to examination of the gastric specimens mailed to public health laboratories, in nearly all cases exceeds the critical period determined by Kramer.[5] "Virulent tubercle bacilli in human sputum after incubation in normal human gastric juice for 10 to 21 hours at 37 C. were rendered nonpathogenic for guinea pigs, and could not be recovered from their organs at autopsy."

Because of concern over possible "false negative" culture reports of mailed-in specimens of gastric juice, a study was undertaken to determine the loss of positive results in such specimens, and to find a medium that would preserve the tubercle bacilli in a viable state.

MATERIALS AND METHODS

Six series of gastric specimens were collected at Glen Lake Sanatorium, Oak Terrace, Minnesota, from patients suspected of having early tuberculosis and from tuberculous patients who were candidates for discharge. The specimens were collected in the morning before food or water was ingested. One-half of each specimen was retained at the sanatorium and the other half was transported by automobile to the Minnesota Department of Health Laboratories for these studies. The latter half was again divided into two portions. One portion of each series designated as "part A", was digested immediately after receipt and inoculated into culture media within 4 hours of collection. The other portion of each series, designated as "part B", was treated for 24 or 48 hours, under conditions shown in Table 1, before digestion.

Both portions "A" and "B", prior to inoculation, were digested with 4 per cent of NaOH for 30 to 45 minutes, neutralized with normal HCl and centrifuged. The sediment was inoculated into two tubes of Petragagni's and two tubes of Hohne's medium. The tubes were observed weekly and considered "negative" if no growth appeared after eight weeks' incubation at 37 C.

*Received for publication, March 28, 1949.

DISCUSSION

The gastric specimens included in these studies were collected from patients with suspected early disease or following treatment. The majority, therefore, contained relatively few tubercle bacilli. This type of specimen was chosen intentionally, because it presents a problem to the public health laboratory arising from delays in examination of the specimens, largely occasioned by mail-

TABLE 1

RESULTS OF CULTURING GASTRIC JUICE FOR *M. tuberculosis*

SERIES	TOTAL NO. OF SPECIMENS	POSITIVE CULTURES OBTAINED				
		Part "A" Inoculated within 4 hr. after Collection		Part "B", Inoculation Delayed		
		No.	Per cent	Treatment of Gastric Juice	No.	Per cent
I	51	22	43.1	Held at 4 C. 24 hr.	15	29.4
II	54	14	25.9	150 ml. distilled water added. Held at 4 C. 24 hr.	8	14.8
III	55	19	34.5	Neutralized with 20% $NaHCO_3$. Held at 4 C. 24 hr.	11	20.0
IV	41	16	39.0	Diluted with 150 ml. buffered water. Held at 4 C. 24 hr.	11	26.8
V	90	19	21.1	Solid Na_2HPO_4* added. Kept at room temperature 24 hr.	21	23.3
VI	60	23	38.3	Solid Na_2HPO_4 added. Kept at room temperature 48 hr.	22	36.7

* According to Dr. C. A. Perry, Bureau of Bacteriology, Maryland Department of Health, Annual Report for 1940, this material is prepared as follows:

1. Weigh 200 Gm. anhydrous Na_2HPO_4.
2. Dissolve by heating in 300 ml. distilled water.
3. Add distilled water to make 500 ml.
4. Add 1.5 ml. of hot solution to each gastric lavage bottle.
5. Autoclave bottles with lids loosely on.
6. To induce crystallization after sterilization, place in refrigerator overnight.

This is sufficient Na_2HPO_4 to neutralize approximately 45 ml. of N/10 HCl.

ing. The delay in the mail is seldom less than 24 hours and may be as long as 72 hours. This period has been shown to be ample for inhibitory factors in some gastric lavages to prevent the growth of tubercle bacilli that might be present. This inhibition was demonstrated in from 32 to 42 per cent of the gastric specimens held 24 hours at 4 C. before culturing. In our study, dilution with sterile distilled water, phosphate buffer solution, or neutralization with 20 per cent sodium bicarbonate did not tend to preserve the tubercle bacilli in a viable state.

Although the number of specimens showing positive cultures is too small to

permit conclusions, an apparent increase in percentage of positive cultures was obtained in specimens to which solid Na_2HPO_4 had been added soon after collection. Approximately the same number of positive cultures were obtained after inoculation was delayed 24 or 48 hours. These findings, however, may merely represent the results of sampling, since relatively few organisms were present.

From the results of cultures in Series VI it appears that the addition of solid Na_2HPO_4 to the gastric specimens will enable the laboratory to obtain considerably more positive cultures after as much as 48 hours' delay at room temperatures. If this result is borne out by further experience, "false" negative reports of mailed-in specimens may be partially eliminated. The Na_2HPO_4 is placed in the specimen containers at the laboratory, so that no additional expense or effort is required of the patient or his physician.

SUMMARY

The inhibitory action of gastric lavages for tubercle bacilli under certain conditions has been confirmed. In from 32 to 42 per cent of the specimens there was inhibition of growth of tubercle bacilli when the specimen was held 24 hours at 4 C.

The addition of crystalline disodium phosphate (Na_2HPO_4) to the specimens of gastric juices soon after collection will enable the laboratory to obtain considerably more positive cultures from mailed-in specimens.

REFERENCES

1. Ames, A. M., Culver, W. E., and Nungester, W. J.: A germicidal factor (G-1-r) present in gastric secretions of certain animals including man. J. Bact. (Abstracts), **51**: 266, 1946.
2. Bartle, H. J., and Harkins, M. J.: The gastric secretion; its bactericidal value to man. Am. J. M. Sc., **169**: 373–388, 1925.
3. Bibby, B. G., Hine, M. K., and Clough, O. W.: The antibacterial action of human saliva. J. Am. Dent. A., **25**: 1290–1302, 1938.
4. Floyd, C., and Page, C. G.: The action of artificial gastric juice and duodenal secretions on tubercle bacilli. Am. Rev. Tuberc., **48**: 174–176, 1943.
5. Kramer, C. H.: Effect of human gastric juice on tubercle bacilli with special reference to the diagnosis of active pulmonary tuberculosis. Am. Rev. Tuberc., **53**: 385–392, 1946.
6. Lester, V.: Four years' experience with examination of material obtained by gastric lavage; demonstration of tubercle bacilli. Am. J. Dis. Child., **47**: 322–330, 1934.
7. Piasecka-Zeyland, E., and Zeyland, J.: On the inhibitory effect of human saliva on the growth of tubercle bacilli. Tubercle, **19**: 24–27, 1937.
8. Schwarting, V. M.: Inhibitive effect of gastric lavage on tubercle bacilli. Am. J. Clin. Path., **15**: 234–239, 1945.
9. Schwarting, V. M.: The action of gastric contents on tubercle bacilli. Am. Rev. Tuberc., **58**: 123–128, 1948.
10. Sprick, M. G., and Towey, J. W.: Isolation of *Mycobacterium tuberculosis* from gastric contents neutralized after varying periods. Pub. Health Rep., **61**: 648–651, 1946.
11. Van Kesteren, M., Bibby, B. G., and Berry, G. P.: Studies on the antibacterial factors of human saliva. J. Bact., **43**: 573–583, 1942.

USE OF CLOROX AND TRISODIUM PHOSPHATE IN DEMONSTRATION OF ACID-FAST BACILLI IN SPUTUM*

MAURICE STEINMETZ TARSHIS, M.S., AND WILLIAM G. LEWIS, M.D.

From the Veterans Administration Hospital, Walla Walla, Washington

Because of a need for more efficient and simpler technics than those which were being used in the laboratory of this hospital for the demonstration of acid-fast bacilli in the sputum, a comparative study of various technics was undertaken. In addition to the direct smear technic, the oxalic acid concentration technic,[2] and the sodium hydroxide concentration technic,[5] which were previously used in this laboratory, the following technics were tested: Clorox concentration (Oliver and Reusser[4]), trisodium phosphate concentration (Corper and Stoner[1]) and phenol-autoclave concentration (Muller and Chermock[3]).

PROCEDURE

The direct smear, the phenol-autoclave concentration and the Clorox concentration technics were used for smear only, while the other three technics were used either for smear, culture, or animal inoculation. The material studied consisted of routine 24-hour specimens of sputum, sent to the laboratory for the examination of acid-fast bacilli.

In order to facilitate handling of the sputum, particularly when it was tenacious, about 10 ml. of distilled water was added to each specimen, regardless of its consistency. Sputums which were too tenacious and difficult to separate were not included in this study. After thorough mixing of sputum with water, 30 ml. of the mixture from each specimen was placed in a 50 ml. screw-cap culture tube. The neck of the tube was coated with a layer of Vaseline to prevent leakage and the cap was tightened. The tubes were placed in a wooden block (Fig. 1) having holes just large enough to admit them and were then placed in a serologic shaking machine (Fig. 2) and shaken for 15 minutes in order to promote uniform distribution of the organisms and to facilitate pipetting of the sputum. Five ml. of sputum from each specimen was next pipetted into each of five 50 ml. screw-cap culture tubes and the sputum in each tube was then treated by one of the following methods:

1. *Phenol-autoclave concentration technic.* Five ml. of 5 per cent phenol was added, the tube was firmly capped and shaken for 15 minutes to insure good mixture, then autoclaved at 15 pounds pressure for 15 minutes. After cooling, most of the supernatant fluid was decanted.

2. *Oxalic acid concentration technic.* Five ml. of 5 per cent oxalic acid was added, the tube was firmly capped and shaken for 15 minutes, then placed in a water bath at 37 C. for

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the authors.

Received for publication, January 28, 1949.

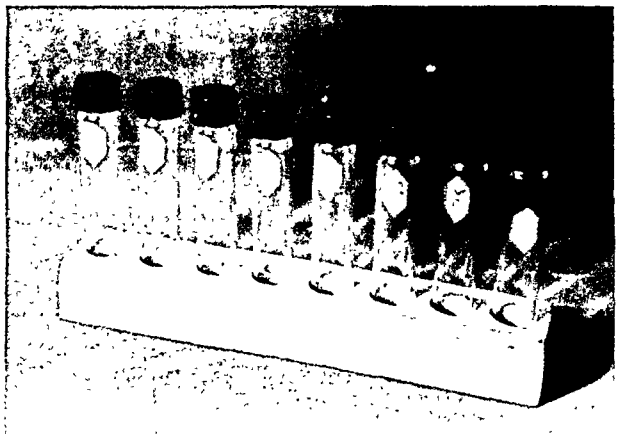


FIG. 1. Wooden block with holes just large enough to hold culture tubes. Block is made to fit snugly into serologic shaking machine.

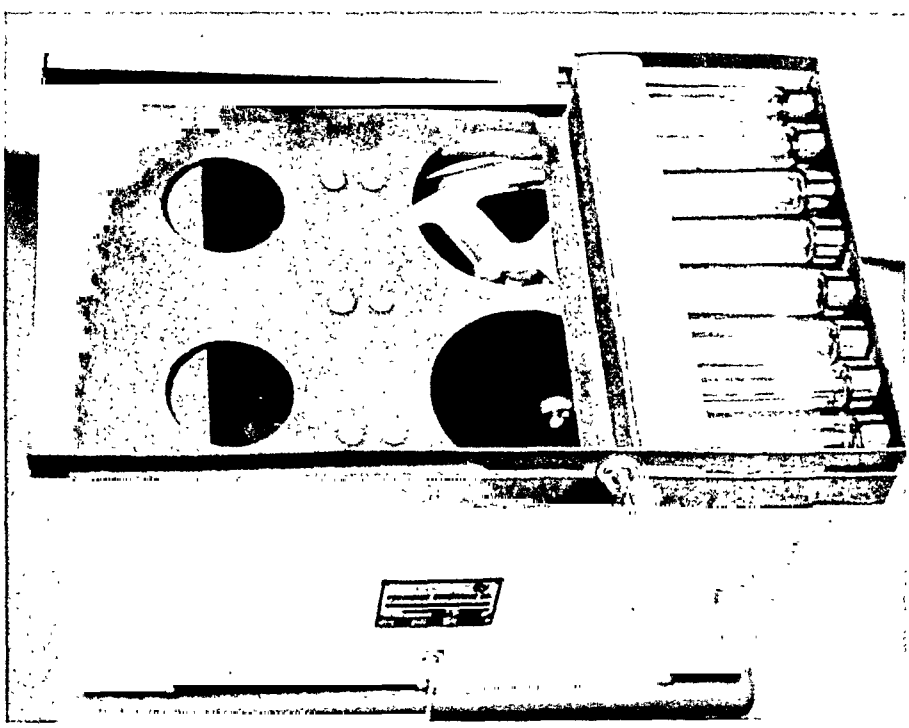


FIG. 2. Wooden block and tubes in place in serologic shaking machine. Caps of screw-cap culture tubes have been tightened to prevent leakage of fluid during shaking.

approximately one hour to digest the specimen. Five-tenths ml. of bromthymol blue indicator was added to the specimen, which was then neutralized with N/1 NaOH.

3. *NaOH concentration technic.* Five ml. of N/1 NaOH was added, the tube was firmly capped, shaken for 15 minutes, then placed in a water bath at 37 C. for approximately one hour to digest the specimen. Five-tenths ml. of bromthymol blue indicator was added to the specimen, which was then neutralized with N/1 HCl.

4. *Clorox concentration technic.* Five ml. of Clorox (active ingredient 5.25 per cent sodium hypochlorite; manufactured by the Clorox Chemical Company, Oakland, California) was added, the tube was firmly capped and shaken for two to five minutes, depending upon the consistency of the sputum.

5. *Trisodium phosphate concentration technic.* Five ml. of C.P. or Analytical Grade of 23 per cent trisodium phosphate ($Na_3PO_4 \cdot 12\ H_2O$) was added, the tube was firmly capped, shaken for 15 minutes, then placed in the incubator at 37 C. for 24 hours to digest the specimen and to destroy contaminants. Following incubation, the specimen was given a preliminary centrifugation at 3000 r.p.m. for 10 minutes, and the supernatant was discarded. Then 5 ml. of physiologic saline was added to the sediment

Following treatment as outlined above in methods 1 to 5, each specimen was centrifuged at 3000 r.p.m. for 10 minutes, the supernatant fluid was discarded, and the sediment smeared on a new, chemically clean slide by means of a cotton-tipped applicator. To enhance fixation of the sediment to the slide, 1 drop of 1:4 dilution of blood serum was added to each sediment and mixed well before making the smears. This was found particularly helpful when there were only a few bacilli in the specimen. Undiluted serum was found to interfere with acid-fast staining.

Direct smears were also made of all specimens before treatment by any of the concentration technics. The smears were made with cotton-tipped applicators which were first dipped into the diluted serum and then into the sputum. All smears were allowed to dry by air, then fixed with heat and stained by the Ziehl-Neelsen technic.

The following method was used for comparison of the degree of positivity by each technic, 100 oil-immersion fields being examined on each slide.

Numerous (5+): Average of over 25 acid-fast bacilli per oil-immersion field.

Many (4+): Average of from 13 to 25 acid-fast bacilli per oil-immersion field.

Moderate (3+): Average of from 3 to 12 acid-fast bacilli per oil-immersion field.

Few (2+): 10 to 250 acid-fast bacilli in 100 oil-immersion fields.

Rare (1+): 1 to 10 acid-fast bacilli in 100 oil-immersion fields.

RESULTS

Five hundred specimens of sputum were examined in this study. Of these, 374 were positive for tubercle bacilli while 126 were negative. The trisodium phosphate technic gave the highest percentage of positive results (75 per cent), followed by the Clorox technic (74 per cent), sodium hydroxide technic (74 per cent), oxalic acid technic (73 per cent) and phenol-autoclave concentration technics (71 per cent), and finally, the direct smear technic (68 per cent).

Table 1 indicates that there was a considerable difference in the degree of positivity obtained by the various technics. The trisodium phosphate technic gave the highest degrees of positivity, followed in order by the Clorox, sodium hydroxide, oxalic acid, and phenol-autoclave technics, and last by the direct smear technic.

The average number of acid-fast bacilli per 100 oil-immersion fields among the positive specimens which were classified as one-plus (1+), for each technic, was

as follows: trisodium phosphate, 9.0; Clorox, 8.3; sodium hydroxide, 7.1; oxalic acid, 5.1; phenol-autoclave, 4.1; and direct smear, 2.1.

Since Clorox kills the tubercle bacillus, it was used only on sputum specimens which were sent to the laboratory for smear only. The Clorox was not permitted to act on sputum for more than five minutes prior to centrifugation, as it was found that prolonged exposure (20 minutes or more) interfered with acid-fast staining. The Clorox technic was considered to be efficient, simple and rapid, and in the smear of the sediment there is little, if any extraneous material present, such as blood cells, fibrous tissue, or other bacteria to interfere with the finding of acid-fast bacilli on microscopic examination.

When cultures or animal inoculations were requested, the trisodium phosphate concentration technic was used, since tubercle bacilli can be exposed to the phosphate at 37 C. for as long as one week without having any significant effect on their viability.

TABLE 1

COMPARISON OF DEGREE OF POSITIVITY BY ALL TECHNICS

TECHNIC USED	1+	2+	3+	4+	5+	TOTAL NUMBER POSITIVE
Direct smear	136	101	75	24	3	339
Phenol-autoclave	112	119	86	31	9	357
Oxalic acid	107	121	97	30	8	363
Sodium hydroxide	88	130	104	31	15	368
Clorox	82	133	106	33	17	371
Trisodium phosphate	76	137	104	41	16	374

The trisodium phosphate technic is efficient and simple, and the sputum specimens can be easily prepared for smear, culture or animal inoculation. This technic has the additional advantage that it is not necessary to complete preparation of the sediments within a limited time after digestion has been started (one to three hours), as in the case of the sodium hydroxide and oxalic acid concentration technics.

Furthermore, sputum concentrated by the trisodium phosphate technic need not be neutralized, thus saving considerable time. With this method, also, there is little extraneous material present to interfere with the finding of acid-fast bacilli on microscopic examination of the smears.

Since trisodium phosphate destroys contaminants in tuberculous sputum, the authors of the technic suggested that the reagent be placed in the receptacles used for collecting the specimens. This immediately prevents the further development of molds and contaminants.

As a result of this study, both the Clorox and trisodium phosphate technics have been used exclusively in this laboratory for digestion and concentration of sputum. Recently we have also applied these technics to the examination of various exudates and body fluids such as urine, spinal fluid, synovial fluid and serum, for examination of acid-fast bacilli with excellent results.

SUMMARY

1. In an effort to determine the simplest and most efficient technics in recovering acid-fast bacilli in sputum, a comparative study of 6 technics was undertaken.

2. Three of the technics (direct smear, phenol-autoclave and Clorox concentration technics) were used for smear only. The other three technics (trisodium phosphate, sodium hydroxide and oxalic acid concentration technics) were used either for smear, culture or animal inoculation.

3. The highest percentage of positive sputums and the largest concentration of acid-fast bacilli were obtained by the trisodium phosphate concentration technic, followed in descending order by the Clorox, sodium hydroxide, oxalic acid, phenol-autoclave concentration technics, and the direct smear technic.

4. On the basis of the results of this study and because of their efficiency and simplicity, the Clorox and trisodium phosphate technics were adopted as the technics of choice in recovery of acid-fast bacilli from sputum.

REFERENCES

1. Corper, H. J., and Stoner, R. E.: An improved procedure for the diagnostic culture of mammalian tubercle bacilli. J. Lab. and Clin. Med., **31:** 1364–1371, 1946.
2. Corper, H. J., and Uyei, N.: Oxalic acid as a reagent for isolating tubercle bacilli and a study of the growth of acid-fast non-pathogens on different mediums with their reaction to chemical reagents. J. Lab. and Clin. Med., **15:** 348–369, 1930.
3. Muller, H. E., and Chermock, R. L.: A rapid staining technique for acid-fast organisms. J. Lab. and Clin. Med., **30:** 169–171, 1945.
4. Oliver, J., and Reusser, T.R.: Rapid method for the concentration of tubercle bacilli. Am. Rev. Tuberc., **45:** 450–452, 1942.
5. Petroff, S. A.: A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J. Exper. Med., **21:** 38–42, 1915.

AN ACCURATE PIPETTING INSTRUMENT FOR SEROLOGIC USE*

ARNE SVEDMYR, MED. LIC.

From the State Bacteriological Laboratory, Stockholm, Sweden

In serial step dilution procedures as applied in serum or virus titration, changing of pipets between steps is essential when accuracy is desirable. Large scale titrations require a considerable supply of graded pipets and calibration errors are easily introduced. More important, however, is the personal factor, brought into prominence in large scale, time-consuming work. In attempts to design an inexpensive semi-automatic pipetting instrument, meeting reasonable demands for accuracy and speed, we arrived at the following device, a short description of which seems justified.

The instrument (see Fig. 1) consists of a constant delivery spring syringe combined with a coupling of the type used for bicycle pumps to which glass pipets can be easily attached.

For use the pipet is introduced into the mouthpiece of the coupling and the handle (A) of the eccentric is pressed towards the barrel whereby the pipet is tightly fitted to the syringe by compression of the rubber gasket (B).

Inasmuch as syringes of glass have proved unsatisfactory, we now use tubes of stainless steel. The nickel-plated brass plunger is tightened by application of a small drop of thin watch oil.

The outer diameter of the pipets should be standardized to fit the coupling. Variation of more than 0.3 mm. in either direction should be avoided. The length of the pipets should be chosen so that the fluid enters only the lower one third to one half. For pipetting of standard amounts of 0.5 or 0.25 ml., a length of 13 cm. has been found satisfactory. This length also permits cotton wool plugging for sterile work. It is important that the opening of the pipets be rather wide, about 1.5 mm., in order to permit an easy flow of the fluid. These ungraded pipets may be obtained at a very low cost.

The syringe is standardized by adjustment of the nuts (C) until the pipet delivers the desired weight of distilled water. In this connection it should be pointed out that the first delivery from a previously dry pipet is less than the standard amount. For this reason the pipet should always be flushed at least once before sampling.

The accuracy in routine pipetting with this syringe is illustrated by the following figures: 37 successive deliveries of distilled water from a syringe standardized at 0.50 ml., pipets being changed each time, showed a mean weight of 502.8 mg. with a standard deviation of ±3.00 mg.

* Received for publication, January 3, 1949.

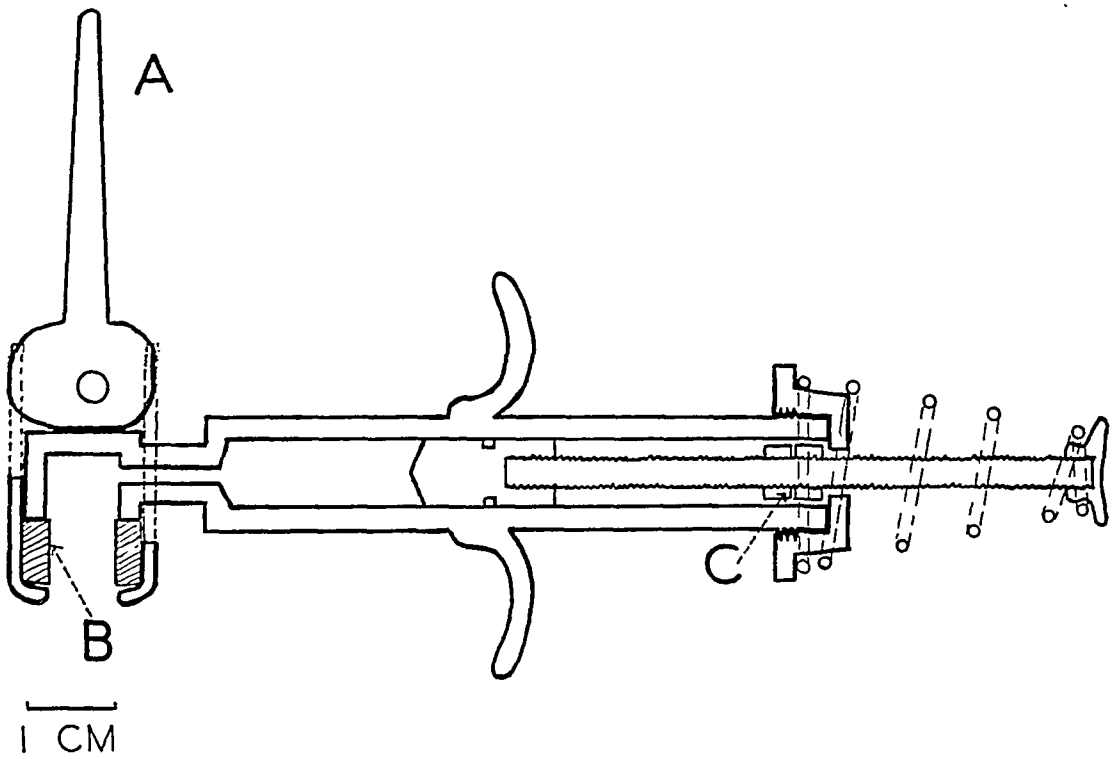


FIG. 1. Pipetting instrument for serologic use

Syringes of this design have been used constantly for one and one-half years without decrease of accuracy and with no need for repair except for changing of worn rubber gaskets. Our instrument has been made by KIFA, Fabriksvägen 9, Hagalund, Sweden.

THE USE OF SILICONE-COATED NEEDLES IN BLOOD DONOR SETS*

TIBOR J. GREENWALT, M.D.

From the Junior League Blood Center of Milwaukee, Inc., Milwaukee, Wisconsin

The silicones are plastics which have water-repellent properties. They are polymers derived from organic silicon compounds. Dri-Film No. 9987†, methyl-chloro-silane, is one of a variety of silicone preparations.

Jacques[1] and his associates reported that whole blood placed in test tubes previously coated with silicone would not coagulate for one and one-half to six hours. It seemed logical to assume that if donor sets were treated with silicone, there would be a reduction in the number of specimens in which clots formed. The report of Rice[2] led us to believe that this assumption was correct. It was felt that coating of the rubber tubing would not be feasible on a large scale and, therefore, only the needles were coated.

METHODS AND EQUIPMENT

The blood was collected into commercially prepared vacuum bottles containing 120 ml. of ACD mixture (citric acid, sodium citrate and dextrose). The donor sets consisted of latex rubber tubing 30 inches long having a bore of $\frac{3}{16}$ inch and a thickness of $\frac{3}{32}$ inch, with a 17-gauge round hubbed needle at the donor-end and a 15-gauge needle at the bottle-end. The flow was controlled by means of a simple screw clamp.

The needles were thoroughly cleaned and dried. The Dri-Film was then forced through the needles by a syringe, paying special attention to the inner surfaces of the hubs. The needles were then rinsed with distilled water to hydrolyze and fix the silicone coating. The hydrochloric acid released by the hydrolysis was neutralized by "syringing" the needles with 4.0 per cent ammonia water. The ammonia water was washed out by copious rinsings with distilled water. The needles were assembled into the donor sets and the sets were autoclaved at 245 F. for 20 minutes.

It was found that the hydrochloric acid fumes released by the hydrolysis of the Dri-Film caused conjunctival irritation, lachrymation, coughing and stinging of exposed skin surfaces. Mild blistering of the skin occurred, necessitating the wearing of latex rubber gloves by the workers. The gloves, needles and other equipment which came in contact with the silicone became so slippery that handling was rendered difficult. The hubs of the needles were so slippery that it was hard to keep them fixed in the ends of the rubber tubing.

* Received for publication, March 3, 1949.

† Supplied by the General Electric Corporation, Schenectady, New York.

RESULTS

Blood was drawn from 359 donors using silicone-treated needles. The same number of bloods were drawn as controls using untreated needles both preceding and following this test. The results are summarized in the following table:

NO. OF DONORS	CONDITION OF NEEDLES	BOTTLES WITH VISIBLE CLOTS	PARTIAL BLEEDINGS
359	Before silicone was used	1	1
359	Silicone-treated	5	2
359	After silicone was discontinued	0	1

CONCLUSIONS

The use of silicone-coated needles in our donor-sets resulted in an increase in the number of specimens with visible clots. Because of the conjunctival, bronchial and skin irritation caused by the hydrochloric acid that was released during the process and because of the slipperiness imparted to the needles and other equipment by the silicone, the procedure was considered to be impractical.

Acknowledgment. The author wishes to thank Dr. Armand J. Quick of the Marquette University School of Medicine for his helpful advice.

REFERENCES

1. JAQUES, L. B., FIDLAR, E., FELSTED, E. T., AND MACDONALD, A. G.: Silicones and blood coagulation. Canad. M. A. J., **55**:26–31, 1946.
2. RICE, W. G.: Use of silicone-treated needles in blood donation. Am. J. Clin. Path., **18**:752–753, 1948.

AN IMPROVED BLOOD CULTURE BOTTLE UTILIZING A SOLID-AND-LIQUID MEDIUM*

RICHARD M. MARWIN, PH. D.†

From the Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis, Minnesota

Since Castaneda's[1] work on a solid-and-liquid medium for the isolation of Brucella from the blood, little progress has been made in adapting the method for routine blood cultures. A possible difficulty with the above device is that the agar may become loosened from the side of the bottle. The device to be described appears to overcome this problem.

METHODS

The bottles are the clear, wide-mouth, screw-neck, French square type, 17 cm. high and 7 cm. wide, with a capacity of 510 ml. The screw-cap metal cover has a 6 mm. hole in the center. Two gaskets were inserted into each cover. The first was made of synthetic or natural soft rubber, approximately 1 mm. thick and was placed next to the cover. Against this, a water-proofed cardboard gasket was inserted as a support for the rubber gasket.

The medium used included a 3 per cent tryptose phosphate, 0.2 per cent yeast extract agar (Difco) and a tryptose phosphate, 0.2 per cent yeast extract broth (Difco). The agar was dispensed in the molten stage over the surface of one of the sides in a layer approximately 10 mm. thick. The agar was made adherent by grinding one entire inside surface of the bottle with a carborundum glass-grinding point attached to a dental drill. Without the ground glass surface, the agar would not adhere satisfactorily to the side of the bottle, regardless of the percentage of agar employed. Before sterilizing the agar, the metal cover was applied loosely and a # 20 gauge needle, 10 mm. in length, was inserted obliquely between the metal cover and rubber gasket in such a manner that it would not penetrate the rubber seal. It was found advisable not to puncture the rubber gasket, as a permanent hole would result during sterilization.

In addition, a sheet of Koroseal‡ was placed over the entire cover and neck of the bottle. A string was tied tightly around the cap to prevent contamination of the needle and cover, and a rubber band was placed around the neck to prevent contamination after sterilization. The unit was then sterilized at 15 pounds of pressure, 121 C., for 20 minutes. Upon removal from the autoclave, the bottles were placed on their sides with the ground glass surface downward and the agar was allowed to harden. Broth prepared beforehand in sterile screw-capped bottles was then added aseptically under ultra-violet light by removing the rubber band around the neck of the bottle and dispensing the broth to a depth

* Received for publication, January 19, 1949.

† Present address: Department of Bacteriology, University of North Dakota Medical School, Grand Forks, North Dakota.

‡ Obtainable from B. F. Goodrich Co., Akron, Ohio.

of 4 cm. (125 ml.). The completed medium was then incubated for three days with intermittent washing of the agar surface each day to detect contaminants. Such a container provides a solid vehicle for the demonstration of organisms growing in the broth. It was possible to maintain aerobic and microaerophilic conditions as well as carbon dioxide tensions with this apparatus. Long periods of storage (15 months or more) have no effect on the quality of either the agar surface or the broth. The bottle with prepared medium is shown in Figure 1.



FIG. 1.

The blood culture sample was obtained in the usual way and the needle removed from the syringe. The Koroseal sheet was then loosened (but not removed) and used to force the needle through the rubber diaphragm. The sheet was then removed and the blood-filled syringe inserted into the hub of the short needle, and the contents injected into the medium. The needle and syringe were then removed and the bottle incubated.

Organisms growing in broth, especially when blood obscures the medium, cannot be demonstrated except by removing samples and staining. This method permits observation of colonies on the agar surface after washing the surface with the broth. This eliminates the necessity of repeated needle punctures or opening of the apparatus. Fogging of the inside glass surface produced by changes of temperature can be cleared by washing the affected portion with the broth. Blood agar may be used for the study of hemolytic activity.

SUMMARY

An improved method of blood culture using a solid-and-liquid culture medium is described. This has been used in one of the larger hospitals and has been found to be simple and practical.

REFERENCE

1. CASTANEDA, M. R.: A practical method for routine blood cultures in brucellosis. Proc. Soc. Exper. Biol. and Med., 64: 114–115, 1947.

THE DETERMINATION OF BROMSULPHALEIN IN THE PRESENCE OF HEMOLYSIS, TURBIDITY AND BILE*

ANDREW FREY AND MARGARET FREY

From the Westchester County Department of Laboratories and Research, Valhalla, N. Y.

The bromsulphalein test is ordinarily a simple and dependable test of liver function. Errors in the test, however, may result from hemolysis, turbidity or the presence of bile in the blood samples.

In the usual methods for bromsulphalein determinations comparator blocks are used. The serum to be tested is divided into two parts. Alkali is added to Part 1 to bring out the purple color of the dye, but if any hemoglobin (from hemolysis) is present it is converted to alkaline hematin which is brown. To overcome this brown interference, an equivalent amount of acid is added to Part 2 (the blank). This converts the hemoglobin to acid hematin which is also brown. The dye remains colorless here. These tubes are now read in the comparator block with the acid blank behind the bromsulphalein standard and a tube of distilled water behind the unknown. The presumption is that the brown of the acid hematin is the same shade and intensity as the brown of the alkaline hematin. Experiments in our laboratory have proved that this is not so. The acid hematin is darker and absorbs more light than the alkaline hematin. Moreover, it is necessary to prepare one such blank tube for each specimen tested.

Experience has shown that it is impossible to detect subtle changes in the purple bromsulphalein in the presence of much hemoglobin, and one still has to contend with turbidity and bile.

We have been able to overcome all of the above difficulties simply by adding two parts of 80 per cent acetone to one part of the serum, mixing, and centrifuging. This treatment precipitates all proteins, including hemoglobin. Turbidity is also removed by the acetone and subsequent centrifugation. No dye is lost. Bile will remain in solution, but will be constant in all specimens. This permits the use of one blank for all specimens. When one drop of 10 per cent alkali is added to each specimen, it will be crystal clear and even slight changes in bromsulphalein concentration can be determined accurately. Either a photoelectric colorimeter or a comparator block may be used for the final readings.

* Received for publication, April 19, 1949

A SOURCE OF ERROR IN BROMSULPHALEIN TESTS*

PAUL JERNSTROM, M.D., AND ELLA PERKINS, M.S.

From the Clinical Laboratories of Jefferson Medical College Hospital, Philadelphia, Pennsylvania

Recently, using Gaebler's [1] method for determination of bromsulphalein, we obtained abnormally high percentages of dye retention in 30 persons in whom other liver function tests were normal.

After careful investigation it was found that the needles which had been used for injection of the dye and for the collection of the blood had been allowed to remain for long periods of time in 70 per cent denatured alcohol tinted with methylene blue, and that in autoclaving the needles some of the methylene blue was precipitated in their lumens. A solution of acid alcohol used to rinse these needles, alkalinized and analyzed photoelectrically, contained the equivalent of appreciable degrees of bromsulphalein retention in serum. According to the U. S. Pharmacopeia, "the addition of sodium hydroxide T. S. to a solution of methylene blue changes the color to a purplish shade." This color is similar to that of serum containing bromsulphalein dye as in Gaebler's test.

REFERENCES

1. GAEBLER, O. H.: Determination of bromsulphalein in normal, turbid, hemolyzed, or icteric serums. Am. J. Clin. Path., **15**: 452–455, 1945.

* Received for publication, March 30, 1949.

short
infusion time...

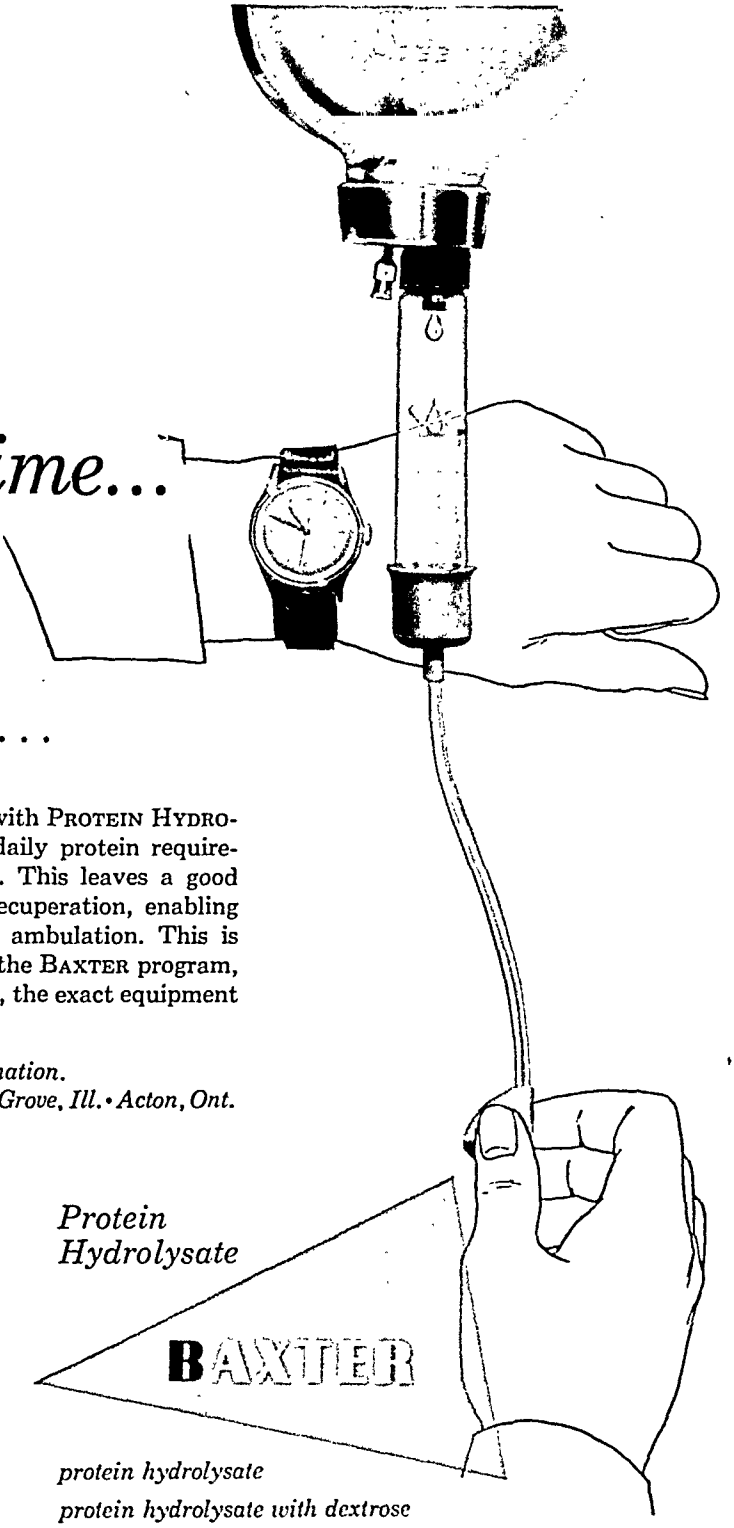
MEANS

more rest time...

earlier ambulation...

The high rate of infusion possible with PROTEIN HYDROLYSATE, BAXTER, provides total daily protein requirements in from two to four hours. This leaves a good portion of the day for rest and recuperation, enabling the patient to benefit from early ambulation. This is another example of the benefits of the BAXTER program, which provides the specific solution, the exact equipment for any parenteral requirement.

WRITE for booklet giving full information.
BAXTER LABORATORIES, Morton Grove, Ill. • Acton, Ont.





Protein Hydrolysate

BAXTER

protein hydrolysate
protein hydrolysate with dextrose
protein hydrolysate with dextrose and alcohol

Available only in the 37 states east of the Rockies (except El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION
General Offices: Evanston, Illinois

(In writing to advertisers, please mention the Journal—it helps.)

Surgical Pathology

By PETER A. HERBUT, M.D.

Professor of Pathology, Jefferson Medical College, Philadelphia, Pa.

VIRTUALLY written in the amphitheatre, this new book is a completely up-to-date surgical pathology in which each part of the body is discussed separately, with sound authority. It is of particular importance to physicians interested in surgery, especially those preparing to take certifying surgical board examinations; to surgical pathologists who want salient facts without the need of wading through a mass of irrelevant detail; and to medical students. For all concerned, two aims have been adhered to: (1) to treat the subject from a regional viewpoint and (2) to summarize, so far as possible, the newer ideas as found in current literature.



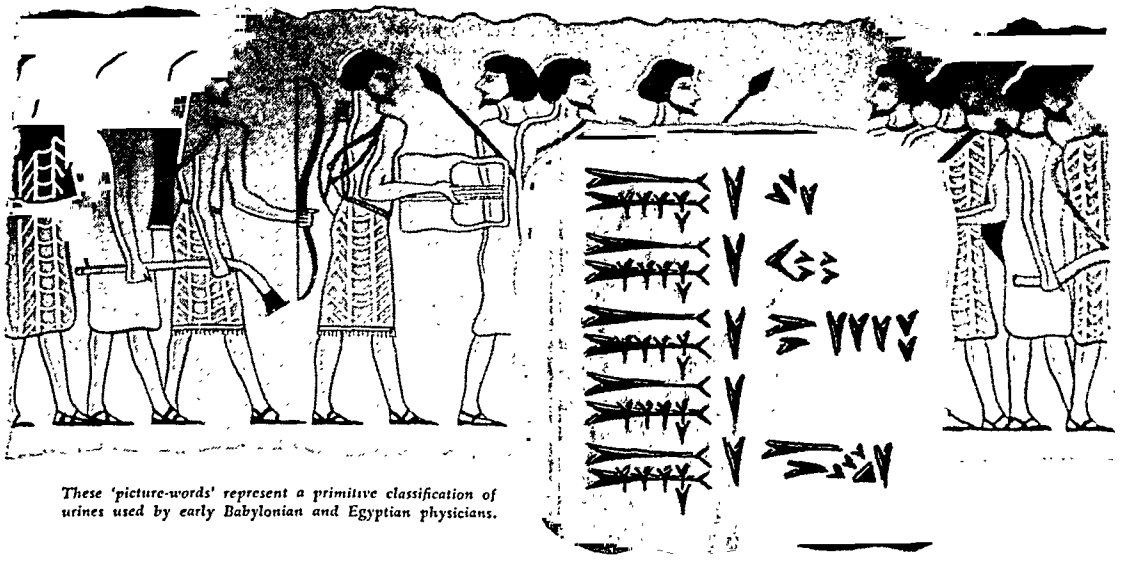
Gastrojejunocolic fistula. One end of the applicator is in the stomach (S) while the other end is in the colon (C). The jejunum (J) has been opened.

With the development of the many specialties in the field of medicine and with the advent of the certifying boards, the surgical pathologist is frequently approached by prospective examinees for recommendation of a source from which to study pathology in their respective fields. *Dr. Herbut's book is designed to serve as this source.* The major surgical specialties: Ear, nose, throat, general surgery, urology and gynecology, are adequately covered and each is considered with sufficient detail to give a basic understanding of the subject. In addition, there is a brief consideration of the embryology, anatomy and histology of each system because of their importance in gaining a thorough understanding of pathology. This is one of the most practical and useful books yet published on surgical pathology.

710 pages. 410 illustrations. $12.00

(Published October, 1948)

LEA & FEBIGER WASHINGTON SQUARE PHILADELPHIA 6 PA.



These 'picture-words' represent a primitive classification of urines used by early Babylonian and Egyptian physicians.

centuries to perfect seconds to perform

When Sumerian and Babylonian physicians, *circa* 4000 B.C., noted the varying colors and constitutions of the "water of the phallus," they were probably not the first uroscopists in history. They were assuredly not the last, for fifty-odd centuries were to elapse before Fehling's first paper on the copper reduction test for urine-sugar appeared in 1848.

But centuries to perfect diagnostic procedures are condensed into seconds to perform the reliable *Clinitest*® method for urine-sugar levels. From start to finish, the test takes less than a minute. This tablet method is simplicity itself . . . readily learned by every diabetic patient. External heating is uniquely eliminated by the *Clinitest* procedure. Routine test interpretation is made easy.

AMES

Clinitest

for urine-sugar analysis

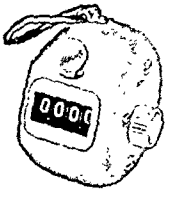
AMES COMPANY, INC • ELKHART, INDIANA

(In writing to advertisers, please mention the Journal—it helps.)

Veeder-Root Hand Tallies for Blood Count



These quick, accurate instruments, used in blood analysis, are available in two models, the double and single, both used for differential and red and white count. When held cupped in the hand a slight pressure on operating lever registers one additional count. When the totals have been recorded, the figures are quickly returned to zero by turning the reset knob. Used in either hand, the other is left free for adjusting the microscope or tabulating. If not available from your Medical Supply House, order direct using the handy coupon below.



Veeder-Root

World's Largest Manufacturers of Counting Devices

VEEDER-ROOT, INC., HARTFORD 2, CONN. A

Please send the following:

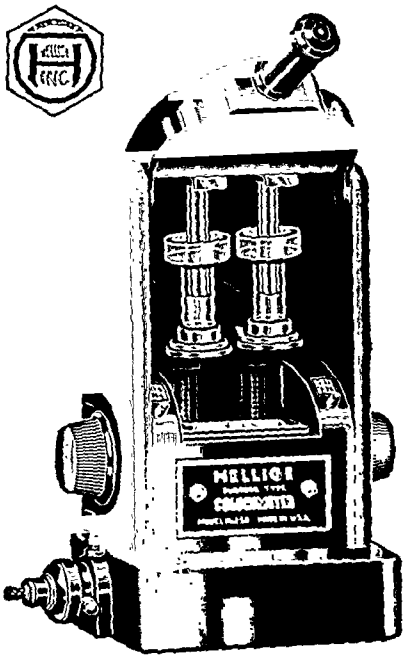
- ☐ Veeder-Root Double Hand Tally Counters (@ $17.00 each)
- ☐ Veeder-Root Single Hand Tally Counters (@ $7.50 each)
- ☐ Descriptive folder on Hand Tally Counters

Institution.....

Street.....*City*.....*State*.....

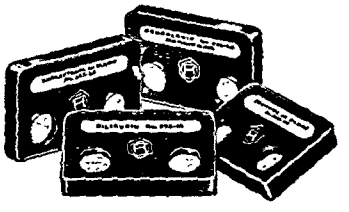
Name.....

HELLIGE-DUBOSCQ COLORIMETER



Closely approaching the theoretical ideal, this universal instrument offers the unusual combination of CONVENIENCE, PRECISION AND DURABILITY.





Hellige Non-Fading Glass Color Standards are available for all popular bio-chemical tests. Unlimited in length of service, these permanent standards quickly repay the small original investment by savings in time and chemicals.

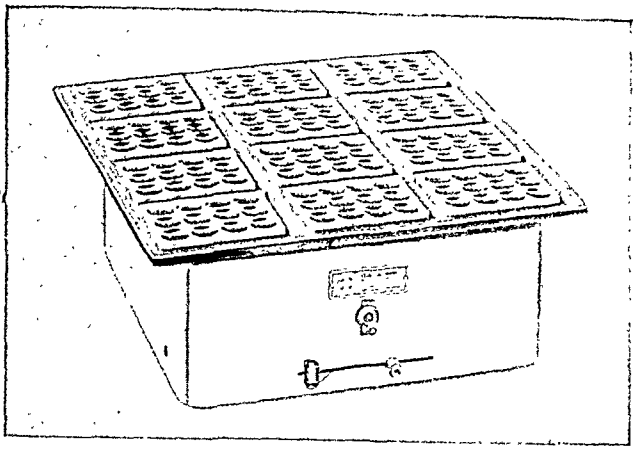
Write for Bulletin No. 250

HELLIGE

INCORPORATED

3718 NORTHERN BLVD., LONG ISLAND CITY 1, N.Y.

HEADQUARTERS FOR COLORIMETRIC APPARATUS



A-2270
and
A-2271

to simulate hand mixing

YANKEE ROTATORS

for **V.D.R.L. (Cardiolipin) test for syphilis, Boerner-Jones-Luken, Mazzini, Kline tests Blood banking—Other mixing operations**

Yankee Rotators simulate hand mixing by imparting a gentle circular motion in the horizontal plane. They are therefore valuable for microslide agglutination tests, blood typing and grouping tests, mixing solutions in flasks, beakers, etc. and many other procedures where gentle continuous mixing is indicated. Three types are available, to meet the requirements of different procedures.

A-2271 and A-2276, designed for the V.D.R.L. (Cardiolipin) test for syphilis, rotate at the required rate of 180 RPM.
A-2270 and A-2275, for the Boerner-Jones-Luken, Mazzini, Kline and other microslide agglutination tests, rotate at 130 RPM.
A-2280, for use in blood banking to mix the anti-coagulant with the donor's blood as it is being received, rotates at 100 RPM.

All models except A-2280 have a built-in timer which can be set to shut the motor off automatically after a 2 to 5 minute mixing period; the timer can be cut out of the circuit when continuous operation is desired.

A-2270 **Yankee Rotator, 11x11″ platform, 130 RPM,** with automatic timer; for 110 v., 60 cycle AC..... **Each $52.50**

A-2271 **Yankee Rotator, 11x11″ platform, 180 RPM,** for the V.D.R.L. (Cardiolipin) test for syphilis; with automatic timer; for 110 v., 60 cycle AC..... **Each $52.50**

A-2275 **Yankee Rotator, 9x9″ platform, 130 RPM,** with automatic timer; for 110 v., 60 cycle AC..... **Each $45.00**

A-2276 **Yankee Rotator, 9x9″ platform, 180 RPM,** for the V.D.R.L. (Cardiolipin) test for syphilis, with automatic timer; for 110 v., 60 cycle AC..... **Each $45.00**

A-2280 **Yankee Donor Blood Rotator, 9x9″ platform, 100 RPM,** without automatic timer; for 110 v., 60 cycle AC **Each $40.00**

Order from your Surgical Supply Dealer

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

(In writing to advertisers, please mention the Journal—it helps.)

REACTION TO INJURY

Pathology for Students of Disease

Based on the functional and morphological responses of tissues to injurious agents.

by WILEY D. FORBUS, M.D.

Professor of Pathology, Duke University: Pathologist to the Duke Hospital

Designed for the use of students and physicians, the book presents and correlates preclinical and clinical data. Distinctive and clinically useful, it combines lucid and accurate writing with more than five hundred illustrations, which tell their own full story. Textbook dogmatism has been avoided. Disease is viewed as the essential reactions of the individual to agents and influences comprising the environment.

816 pp. 532 illus. $9.00

THE WILLIAMS & WILKINS COMPANY

BALTIMORE 2, MD.

Do you have students in training, or employees who need to review?

Then you will want to use as a guide, Doctor I. Davidsohn's CURRICULUM FOR SCHOOLS OF MEDICAL TECHNOLOGY.

A few of its excellent features are:

Introduction, with invaluable advice and suggestions for both students and teachers.

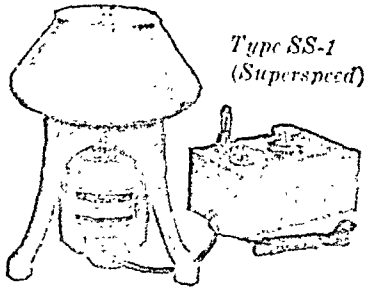
Time schedule for training in each department, adaptable to courses from twelve to twenty-four months in length.

Revised list of references for study and collateral reading in each phase of the training course.

Price for Approved Schools, $0.50; for other buyers, $1.75.

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
American Society of
Clinical Pathologists
Muncie, Indiana

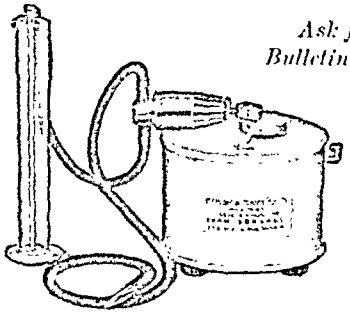
SERVALL
ANGLE CENTRIFUGES
Ask for Bulletin J-71
with complete data on all models



Type SS-1
(Superspeed)

With safety design self-centering device and dynamical balance. 13,000 r.p.m. 20,000 × G. Cap. 8 × 50cc or 15cc tubes.

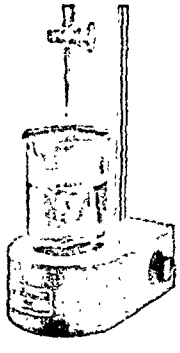
NEW TYPE SS-2 VACUUM CENTRIFUGE
16,000 r.p.m. 45,000 × G.
Capacity 16 × 50cc or 15cc tubes



Ask for Bulletin J-72

STERLING AUTOMATIC
AND
HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.
Easy to Operate—Time Saving



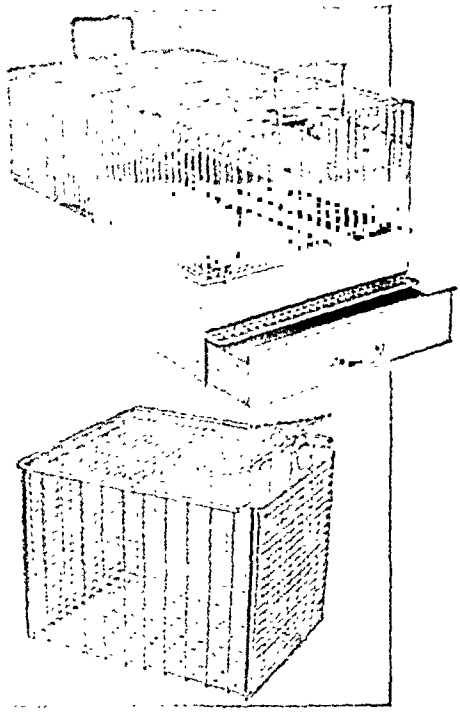
SERVALL
Magnetic Stirrer

Ask for Bulletin J-74

For stirring in open vessels or in closed systems under any pressure or under sterile conditions. Makes possible central rotation with vortex formation, epicyclic motion without vortex, simultaneous stirring in several small containers, etc.

Manufacturer and Distributor:
IVAN SORVALL, Inc.
210 FIFTH AVE.-NEW YORK 10, N. Y.

FOR THE MOST DISCRIMINATING TECHNICIAN



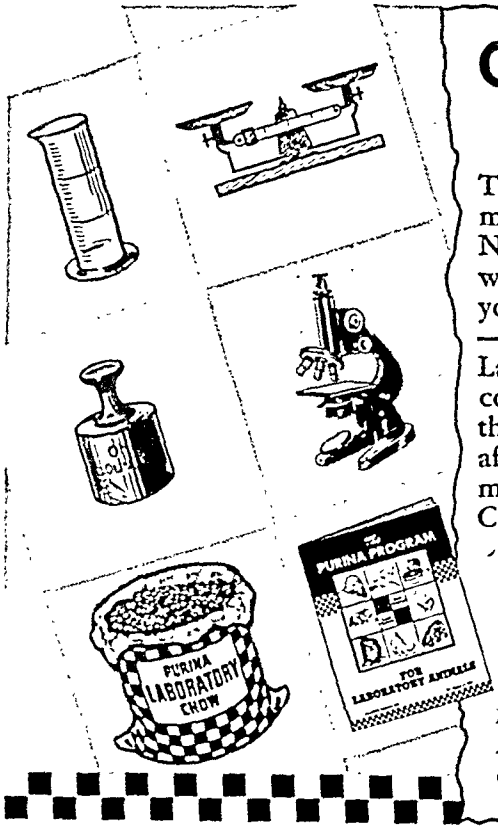
Laboratory Animal Cages by *Bussey*



Bussey cages are the ultimate in cleanliness and ease of servicing. Several specific-purpose cages, one all-purpose cage...for guinea pigs, hamsters, rat colonies, small rabbits and cats. Racks designed for each type of cage. Cages are suspended, with dropping pans as separate units. Finest electric welded construction. Finish specially resistant to strong cleansing and disinfectant solutions. Write for literature and prices. Free consultation on special equipment problems. Write today.

Prompt Delivery

BUSSEY PRODUCTS CO.
6000-58 W. 51st St. • Chicago 38, Ill.



CONSTANTS YOU CAN *Count On*

They're part of your necessary equipment... they're standard, dependable. No laboratory work can be carried on without them! In the field of nutrition you need a constant you can count on—an unchanging factor like Purina Laboratory Chow. It's a ration with a constant formula... the same food in the same form day after day, month after month—to make your experiments more valuable. Try Purina Laboratory Chow—a constantly uniform ration.

RALSTON PURINA COMPANY • St. Louis 2, Mo.

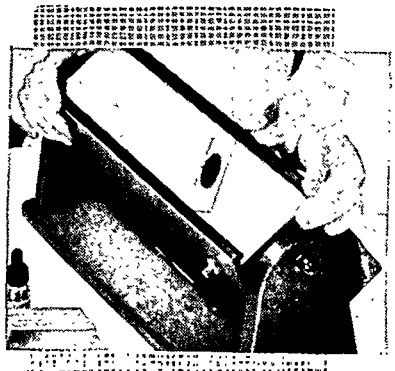
RALSTON PURINA COMPANY
1706 Checkerboard Square, St. Louis 2, Mo.
Please send me your new 28-page handbook (SP4629) on laboratory animal nutrition.

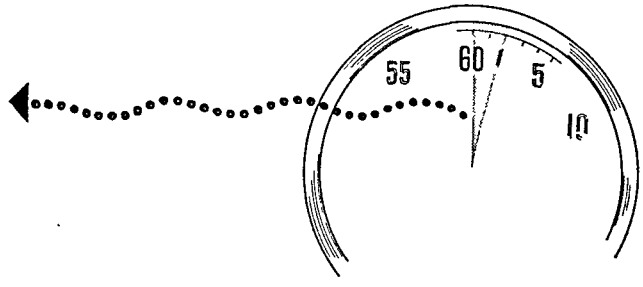
*Name*_____
*Address*_____
*City*_____ *State*_____
(Handbook available only in U. S.)

TWO drops of blood

ONE drop of anti-Rh serum

. . . Test complete in TWO minutes or less

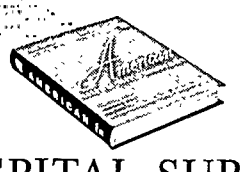




Rapid, accurate, satisfying results in blood testing are a certainty with **Rh** serums from AMERICAN. No microscope is needed. The Slide Method gives clear, distinct, unmistakable readings to the naked eye. Clumping starts in 45 seconds—is complete in from 1 to 2 minutes.

These potent and stable serums may be used with fresh, clotted, oxalated, heparinized or citrated blood. Serums are prepared from immunized *human* donors in a Government licensed laboratory—in strict accord with NATIONAL INSTITUTE OF HEALTH specifications.

WRITE TODAY *for descriptive literature on* AMERICAN'S *complete line of blood-typing and blood-grouping serums.*



PLAN WITH AMERICAN
. . . the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS

LOWER PRICES

Effective March 1st, 1949

Standardized Desiccated Complement

by Cappel

3 cc	$2.50
5 cc	3.75
7 cc	5.00
15 cc	9.00

10% discount on lots of 50 or more

All reagents for the Kolmer-Wassermann Test, produced *by Cappel*, are standardized against each other. The titre for each reagent is listed on the direction sheet which is enclosed with each ampul. For this reason we recommend the use of the following reagents:

by Cappel

KOLMER-WASSERMANN ANTIGEN (STANDARD)
KOLMER-WASSERMANN ANTIGEN (NEW)

ANTI-SHEEP HEMOLYSIN (GLYCERINATED)
(*Equal parts of Glycerin and Hemolysin*)

POOLED SHEEP CELLS
(*Washed, stabilized, 10% suspension*)

STERILE SHEEP BLOOD (CITRATED)
STERILE SHEEP BLOOD (ALSEVER'S)
(*Equal parts of blood and modified Alsever's solution*)

POSITIVE SYPHILITIC SERUM (4+)
(*Desiccated*)

CAPPEL LABORATORIES
Diagnostic Reagents
Box 352 Wayne, Pa.

WHY YOU SHOULD SPECIFY

"VACSEAL"

GUINEA PIG COMPLEMENT

Because it is a superior complement—the result of a decade of experience in guinea pig complement production, the proper choice and preparation of animals and the use of improved technics.

Because our large output enables us to pool the serum of no less than 100 guinea pigs per lot, assuring a more uniform titer. It also makes it possible for you to obtain a complete lot of any complement which has proved, through pretesting, to be particularly desirable for your purpose.

Because of the high vacuum and all glass containers, storage for at least two years is possible. To you this means economy and convenience.

"Vacseal" Complement is distributed in three sizes with a special diluent for restoration to the following amounts:

3 ml. 7 ml. 20 ml.

Any recognized laboratory will receive a trial 3 ml. sample upon request.

CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK

BBL

CULTURE MEDIA

FOR THE

ASSAY OF ANTIBIOTICS

Penicillin Streptomycin

Tyrothricin

Literature and prices sent on request

BALTIMORE BIOLOGICAL LABORATORY
1640 Gorsuch Avenue Baltimore-18, Md.

MICHAEL REESE HOSPITAL
POSTGRADUATE SCHOOL

HEMATOLOGIC DIAGNOSIS

August 1–13, 1949 (Full-Time)

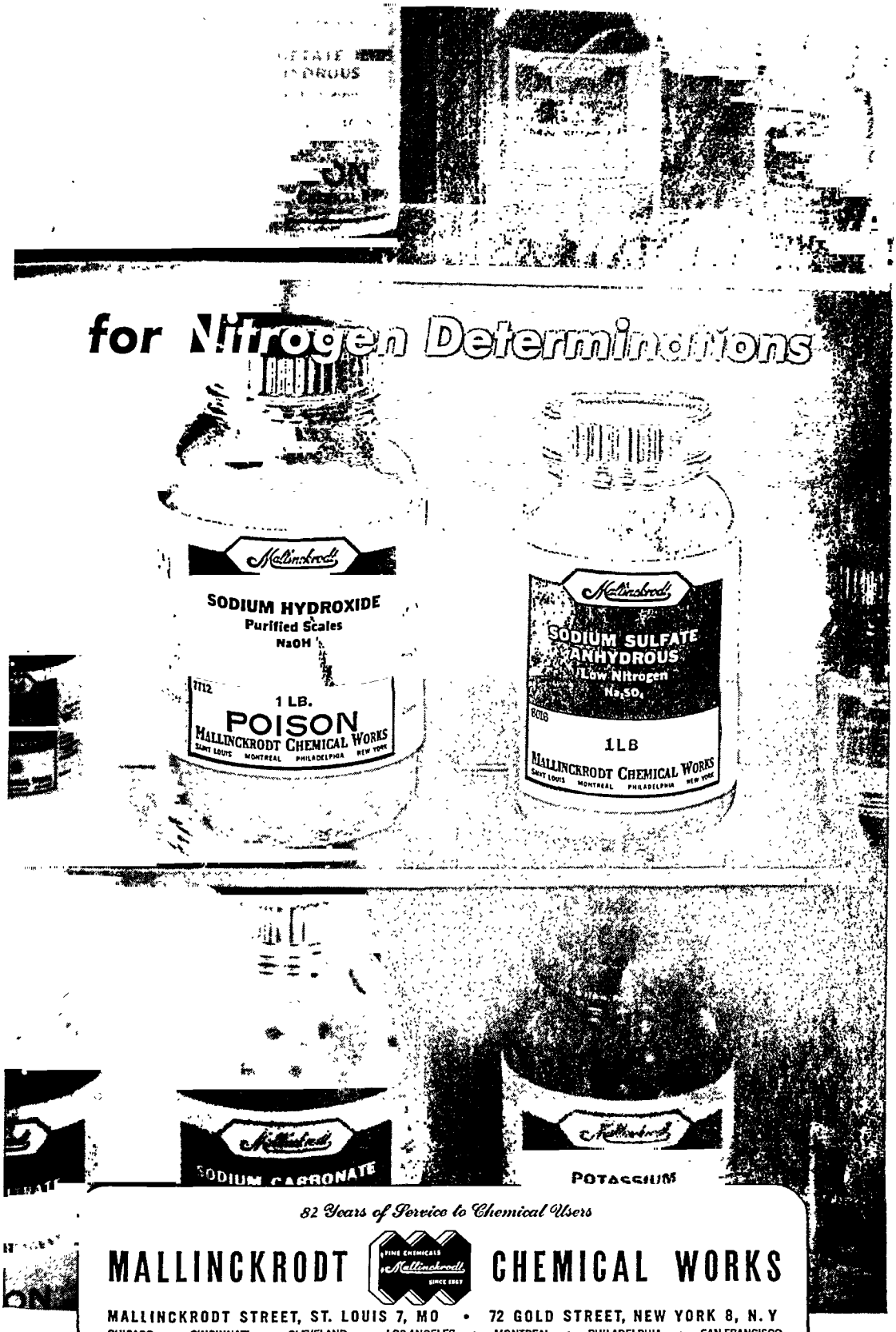
By

KARL SINGER, M.D.
Director of Hematologic Research

Instruction in actual reading of slides—normal and pathological specimens—Peripheral blood and bone marrow. Individual slide collection provided and may be retained by student. Review of present status of Hematology.

TUITION: $100.00

For further information, address:
Dr. Samuel Soskin, Dean
Michael Reese Hospital
Postgraduate School
29th St. & Ellis Avenue
Chicago 16, Illinois

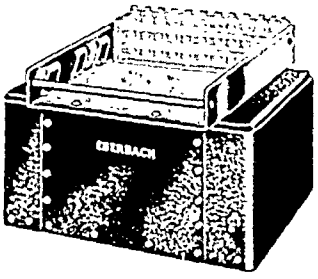

for Nitrogen Determinations
SODIUM HYDROXIDE
Purified Scales
NaOH
1 LB.
POISON
MALLINCKRODT CHEMICAL WORKS
SODIUM SULFATE
ANHYDROUS
Low Nitrogen
1LB
MALLINCKRODT CHEMICAL WORKS
82 Years of Service to Chemical Users
MALLINCKRODT CHEMICAL WORKS
MALLINCKRODT STREET, ST. LOUIS 7, MO • 72 GOLD STREET, NEW YORK 8, N. Y
CHICAGO • CINCINNATI • CLEVELAND • LOS ANGELES • MONTREAL • PHILADELPHIA • SAN FRANCISCO
UNIFORM DEPENDABLE PURITY



WHITE MICE
WHITE RATS



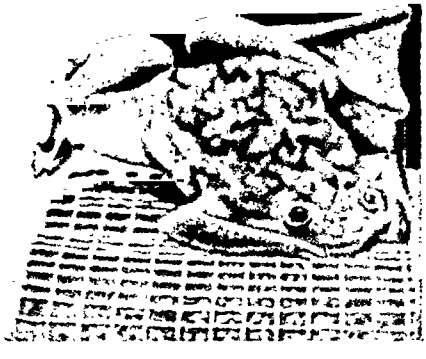
RESEARCH SUPPLY CO.
2436 W. York Street
Philadelphia 32, Pa.



Eberbach 4 Rack
KAHN SHAKERS

"Snap Out" feature permits instant removal or insertion of any rack without adjusting clamps or disturbing other racks. Shaker-action rigidly conforms to specifications of the Kahn Test. Above Shaker # 75-685A sells for $92.00. Write for literature.

Eberbach & Son Company
LABORATORY APPARATUS & SUPPLIES
ANN ARBOR, MICH.



XENOPUS LAEVIS FROG

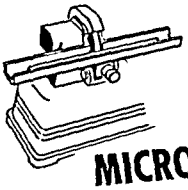
Pregnancy Diagnosis

Animal Costs Greatly Reduced

We assure you of a continuous supply. Accurate, speedy, and inexpensive. Average animal cost per test 20 cents to 35 cents. We carry a complete stock of Laboratory Tanks, Isolation Jars, Holders, and correct reagents and apparatus for running tests Speedy delivery via AIR EXPRESS. Current brochure showing new low prices sent upon request.

Jay E. Cook, Importer

P. O. Box 302 Baltimore 3, Md., U.S.A.
Cable Address—XENOPUS-BALTIMORE



the Berkeley
MICROTOME KNIFE SHARPENER
assures an
INCOMPARABLE EDGE
3 times faster...



COMPARE THE EDGES. Photomicrograph *(a)* shows a microtome knife edge sharpened by the Berkeley sharpener. Note its even smoothness, assurance of better sections. Photomicrograph *(b)* shows a blade laboriously hand sharpened, honed and stropped, but note the nicks and serrations still remaining.

COMPARE THE TIME. In tests, wherever made, and in practice in hundreds of laboratories, the Berkeley Microtome Knife Sharpener gives perfect edges in one-third the time required for hand honing and stropping.

Let us send you Bulletin MKS-14 giving full information about this indispensable laboratory aid.

Berkeley Scientific Company
SIXTH AND NEVIN AVE • RICHMOND, CALIFORNIA

(In writing to advertisers, please mention the Journal—it helps.)

for

BLOOD TYPING
RH TESTING

you need the highest-titred serum available.

ANTI-HUMAN PRECIPITIN SERUM (rabbit)
for the Coombs Test

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human Red Cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic Serum
for control tests

P.S.A.
(Paraffin Section Adhesive)

Write for complete information.

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York

FOR BEST RESULTS USE

• USSLA •

PRODUCTS

*

Krautman's

Albu-Mi-Test

TABLETS

- **Direct, Rapid and Reliable Method for the Detection of Albumin in Urine.**
- **No Solutions or Reagents**
- **No Measuring**
- **No Heating**

Simple Technique for the Detection of Albumin in Urine

Two Hundred and Fifty Tablets $1.95
Box of Six Bottles less 10%

U. S. Scientific Laboratories

237 Christopher Ave., Brooklyn 12, N. Y.

Advertisers in the Journal Enjoy these advantages

• Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.

• The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.

• Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.

THE NEW

LaMotte WATER-SOLUBLE pH INDICATORS

(Sulfon-phthalein series) pH 0.2—9.6



These products were originally developed in the [illegible] of the same superior [illegible]ators hitherto available.

They have the distinct advantage of being instantly soluble in distilled water, hence are easy to convert to solution form. Use of alkali, as required in the older technic, is eliminated, and no heating is necessary.

The clean quick way to prepare indicator solutions for accurate pH work.

Made exclusively by

LAMOTTE CHEMICAL PRODUCTS COMPANY

Dept. D Towson, Baltimore 4, Md.

If you do not have the LaMotte Blood Chemistry Handbook, a complimentary copy will be sent upon request without obligation.

LABORATORY C&B REAGENTS

Inorganic and Organic Chemicals

•

Biological Stains Solutions

•

Chemical Indicators Test papers

•

Write for copy of our new catalog

The COLEMAN & Bell Co., Inc.,

Manufacturing Chemists: Norwood, O., U. S. A.

COLEMAN & BELL

ANOTHER MOSBY BOOK!

DOCTOR and PATIENT and the LAW

By LOUIS J. REGAN, M.D., LL.B., Member State Bar of California.
SECOND EDITION. 590 Pages. Price, $10.00

DESIGNED TO HELP MEDICAL MEN IN UNDERSTANDING THE LAW AND TO GOVERN AND PROTECT THEMSELVES UNDER IT

Law, like medicine, is not a static but a dynamic practice. The law must and does change to meet new circumstances, new requirements of society. Similarly, those engaged in practicing the healing arts can no longer afford to isolate themselves from the general social and economic currents of the times.

In this new Second Edition, Dr. Regan emphasizes the responsibility of professional groups to society in forming and guiding community thinking and planning in all matters pertaining to health, hospitalization, and medical care.

He discusses the misunderstanding engendered by the great number of unjust malpractice suits which has tended to break down the confidence of the public in the healing professions and how this public ill-will may be used destructively against the professions.

He stresses the responsibility of the patient himself in the doctor-patient relationship and the need for public education on that point. However, since there are few actual cases of malpractice, this book will certainly help any professional man to fully understand the law and how to govern and protect himself under that law.

THIS EDITION HAS BEEN ENLARGED BY A NUMBER OF NEW SECTIONS:

- Adoption
- Adverse Party Called as Witness
- Artificial Insemination
- Blood Grouping Tests in Affiliation Proceedings
- Contracts in Restraint of Trade
- Coroners and Medical Examiners
- Disclosures Under Statute
- Dying Declarations
- Essentials of a Constructive Malpractice Program
- Federal Food, Drug, and Cosmetic Act
- Insane Persons

Just Released!—Scobee's A CHILD'S EYES
A book written for the parents of children with crossed eyes —answering all their questions with sincerety, sympathy and simplicity. Useful to anyone treating child patients with this affliction. 109 pages. Price $2.00

ORDER FORM

The C. V. Mosby Company
3207 Washington Blvd.
St. Louis 3, Missouri

AJCP 7-49

Please send me: Regan's DOCTOR AND PATIENT AND THE LAW—The price is $10.00
Scobee's A CHILD'S EYES—The price is $2.00
☐ Enclosed find check. ☐ Charge my account.

Name _____

Address _____

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia...O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Benbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

American Society of Clinical Pathologists

OFFICERS

President: Dr. O. A. Brines, Wayne University College of Medicine, Detroit 26, Michigan
Vice-President: Dr. C. H. Manlove, 5725 S.E. Stark Street, Portland 15, Oregon
President-Elect: Dr. J. B. McNaught, University of Colorado School of Medicine, Denver, Colorado
Secretary-Treasurer: Dr. C. G. Culbertson, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana

STANDING COMMITTEES

EXECUTIVE COMMITTEE

W. S. Thomas, Chairman, Rochester, N. Y.
T. J. Curphey, Long Island, N. Y.
F. W. Konzelmann, Atlantic City, N. J.
L. W. Larson, Bismarck, N. D.
S. P. Reimann, Philadelphia, Pa.
J. R. Schenken, Omaha, Neb.

BOARD OF CENSORS

L. W. Diggs, Chairman, Memphis, Tenn.
J. J. Andujar, Fort Worth, Tex.
F. C. Coleman, Des Moines, Iowa
J. W. Howard, Wilmington, Del.
V. M. Johnson, West Palm Beach, Fla.
C. P. Larson, Tacoma, Wash.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. Montgomery, Chairman, Muncie, Ind.
A. G. Foord, Pasadena, Cal.
F. B. Lynch, Jr., Philadelphia, Pa.
F. B. Queen, Portland, Ore.
R. G. Stillman, New York, N. Y.
A. H. Wells, Duluth, Minn.

APPOINTED COMMITTEES 1948-1949

GENERAL RESEARCH COMMITTEE

M. Strumia, Chairman
B. S. Kline
A. H. Sanford
W. M. Simpson

COMMITTEE ON HEMATOLOGIC TERMINOLOGY

E. E. Osgood, Chairman
L. Berman
R. R. Kracke
F. J. Heck
L. R. Limarzi

BLOOD BANK COMMITTEE

D. H. Kaump, Chairman
L. Y. Dyrenforth
L. W. Larson
F. W. Hartman
R. G. Stillman

COMMITTEE ON REGISTRY OF GENERAL TUMORS

J. W. Kernohan, Chairman
L. V. Ackerman
Col. J. E. Ash
W. A. D. Anderson
S. W. Lippincott
I. Perry

REPRESENTATIVE ON NATIONAL RESEARCH COUNCIL SUBCOMMITTEE ON ONCOLOGY

S. Warren

REPRESENTATIVE ON BOARD OF GOVERNORS OF COLLEGE OF AMERICAN PATHOLOGISTS

T. J. Curphey

COMMITTEE ON SEMINAR

I. Davidsohn, Chairman
P. R. Cannon
J. B. McNaught
Gen. R. O. Dart
R. A. Moore

PROGRAM COMMITTEE

C. G. Culbertson, Chairman
R. A. Moore, Co-Chairman
C. J. Bucher
F. B. Queen
F. W. Sunderman

COMMITTEE ON NECROLOGY

H. R. Brown

COMMITTEE ON EDUCATION AND PUBLICITY

W. O. Russell, Chairman
J. L. Carr
W. M. Germain
E. F. Hirsch
D. A. Nickerson
S. P. Reimann

COMMITTEE TO STANDARDIZE EDUCATION AND TRAINING OF MEDICAL TECHNOLOGISTS

J. J. Andujar, Chairman
F. C. Coleman
E. Moss
O. O. Christianson
M. Oosting
R. Miller
P. Rosenblatt
J. P. Tollman

DIFCO

DETECTION OF THE
Typhoid - Dysentery
GROUP

- **Bacto- S S Agar**

 is a selective medium especially designed for use in isolation of fastidious *Shigella* and *Salmonella* strains. The selective action of this medium restrains to a large extent the development of coliform bacteria with minimum restriction of fastidious strains of the typhoid-dysentery group. Because of the inhibitive action of the medium on coliform bacteria, it is possible to inoculate the medium heavily with feces, thereby greatly increasing the chance of positive isolations from samples containing very few pathogens.

- **Bacto-Bismuth Sulfite Agar**

 is a highly selective medium for isolation of *Salmonella typhosa*. The unusual selective properties of this medium permit the use of large inocula of feces and other suspected material without overgrowth of extraneous intestinal bacteria.

- **Bacto-MacConkey Agar**

 is an excellent differential medium for use in conjunction with Bacto- S S Agar and Bacto-Bismuth Sulfite Agar. This medium supports rapid and luxuriant growth of even the most fastidious strains of the typhoid-dysentery group. Although MacConkey Agar does not inhibit coliform bacteria it does afford excellent differentiation of colonies of pathogens from those of the lactose fermenting bacilli.

- **Bacto-Tetrathionate Broth Base**

 is recommended for the preparation of Tetrathionate Broth to be used as an enrichment medium in the isolation of intestinal pathogens from stools and other suspected material. It is an excellent aid in detection of carriers and in determining the release of patients.

Specify "DIFCO"

The Trade Name of the Pioneers in the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES

DETROIT 1, MICHIGAN

VOLUME 19 JUNE 1949 NUMBER 6

AMERICAN JOURNAL OF CLINICAL PATHOLOGY

OFFICIAL PUBLICATION OF
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R. BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JUORNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members could be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: **The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.**

Agents: For Australia: Angus and Robertson, Limited, 89-95 Castlereagh Street, Sydney; for the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 JUNE 1949 NUMBER 6

CONTENTS

Experimental Ovarian Tumors in Rats. G. R. BISKIND AND M. S. BISKIND.. 501

On the Neurogenesis of So-Called Granular Cell Myoblastoma. J. A. FUST AND R. P. CUSTER..... 522

An Evaluation of the Use of Group-Specific Substances in Group O Blood Transfusions. O. M. ALTON AND W. R. PLATT..... 536

The Diffusely Enlarged Uterus. K. M. TRUEMNER AND D. H. KAUMP..... 544

Observations on the Occurrence of Varices of the Esophagus in Routine Autopsy Material. T. WEINBERG..... 554

Case Reports

Epidermoid Cyst of the Spleen. H. J. LINN AND E. P. ELLIAS..... 558

Simulated Homicide. Obstructive Laryngeal Papilloma in a Child. J. W. SPELMAN 565

Gaucher's Disease in a Negro. R. M. CHOISSER AND R. R. MONTGOMERY 570

Clinicopathologic Conference. B. HALPERT AND S. N. STONE..... 576

Selected Abstracts..... 583

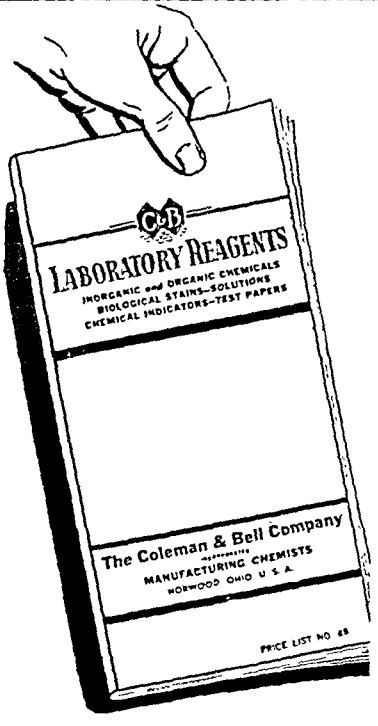
Book Reviews..... 591

Obituaries..... 597

News and Notices..... 598

C&B

LABORATORY REAGENTS



New Catalog FREE

If you have not received a copy of this new catalog please notify us at once.

Inorganic and Organic Chemicals

•

Biological Stains Solutions

•

Chemical Indicators Test Papers

•

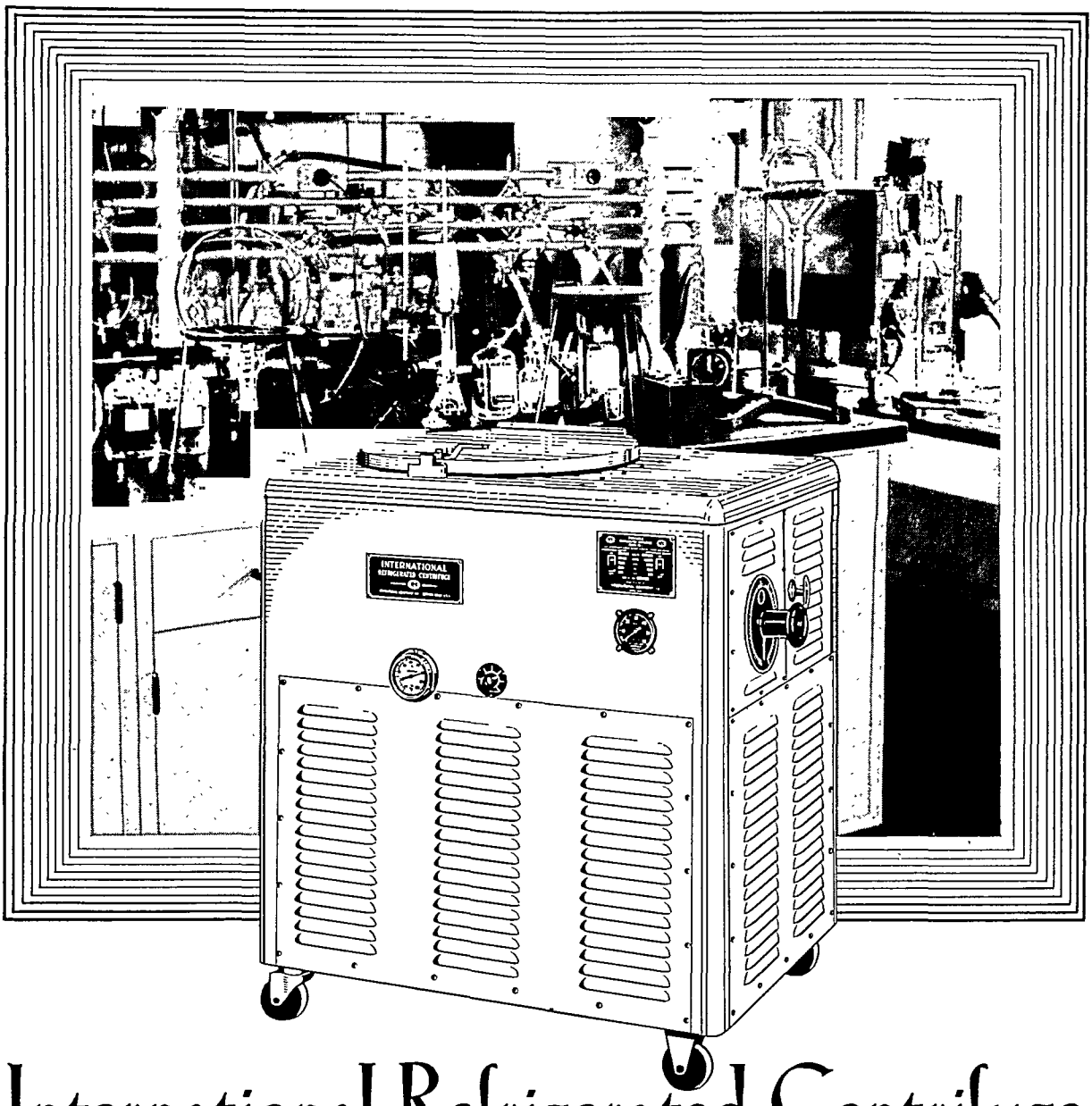
Distributed by
Physician and Laboratory Supply Houses

The COLEMAN & BELL Co., Inc.

Manufacturing Chemists : Norwood, Ohio, U. S. A.

COLEMAN & BELL

(In writing to advertisers, please mention the Journal—it helps.)



International Refrigerated Centrifuge

Centrifugation under controlled temperatures at or well below 0° C. offers new possibilities to the biochemist. The cancer program —virus investigations—poliomyelitis research —enzyme research—low temperature solvent fractionation—all have benefited by the use of this new research tool. Countless other applications are waiting the guidance of the research scientist.

The International Portable Refrigerated Centrifuge, Model PR-1, is now equipped with a larger capacity compressor. Centrifuge speeds and capacities remain unchanged, but much lower controlled temperatures are now possible. For example, with six 100 ml. tubes at 5000 r.p.m., temperatures as low as -15° C. can be maintained; with four 250 ml. bottles at 2600 r.p.m., -8° C.; with four 25 ml. tubes at 18,000 r.p.m., -5° C.

Here is a scientifically designed research instrument definitely worth investigating. Write for descriptive Bulletin RC, 1949.

INTERNATIONAL EQUIPMENT COMPANY
Boston 35, Massachusetts

another **ALOE** exclusive

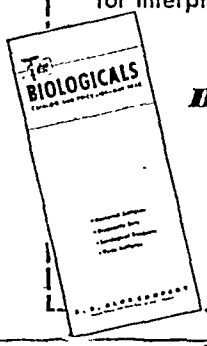


Experience and intensive research bring you Markham Diagnostic Biologicals

The Markham Diagnostic Biologicals . . . virus antigens and anti-sera . . . bacterial antigens and anti-sera . . . serological accessories including preserved sheep cells are now available for more exact confirmatory diagnosis. Each product is prepared to the highest standards and is fully guaranteed as to specificity and titre.

Standard Technique

With virus antigens, the principles of the well known complement-fixation technique are employed. The rapid slide or the macroscopic test tube method is used with bacterial antigens and anti-sera. Each vial is furnished with complete directions including suggestions for interpreting results.



FREE BIOLOGICAL CATALOG

Write for your free copy of complete catalog on Markham Diagnostic Biologicals No. 201.

Virus Antigens Anti-Viral Sera

- Eastern Equine Encephalomyelitis
- Western Equine Encephalomyelitis
- Influenza "A"
- Influenza "B"
- Lymphocytic Choriomeningitis
- Lymphogranuloma Venereum
- Mumps, Epidemic Parotitis
- Psittacosis-Human Pneumonitis Group
- St. Louis Encephalitis
- Rabies

Serological Accessories

- Amboceptor, Anti-sheep Hemolysin
- Guinea Pig Complement
- Washed Standardized Sheep Cells

Bacterial Antigens

- Brucella Abortus
- Brucella Melitensis
- Paratyphoid A
- Paratyphoid B
- Tularense
- Typhoid, H and O
- Preserved Sheep Cells
- *Proteus OX19*

Anti-Bacterial Sera

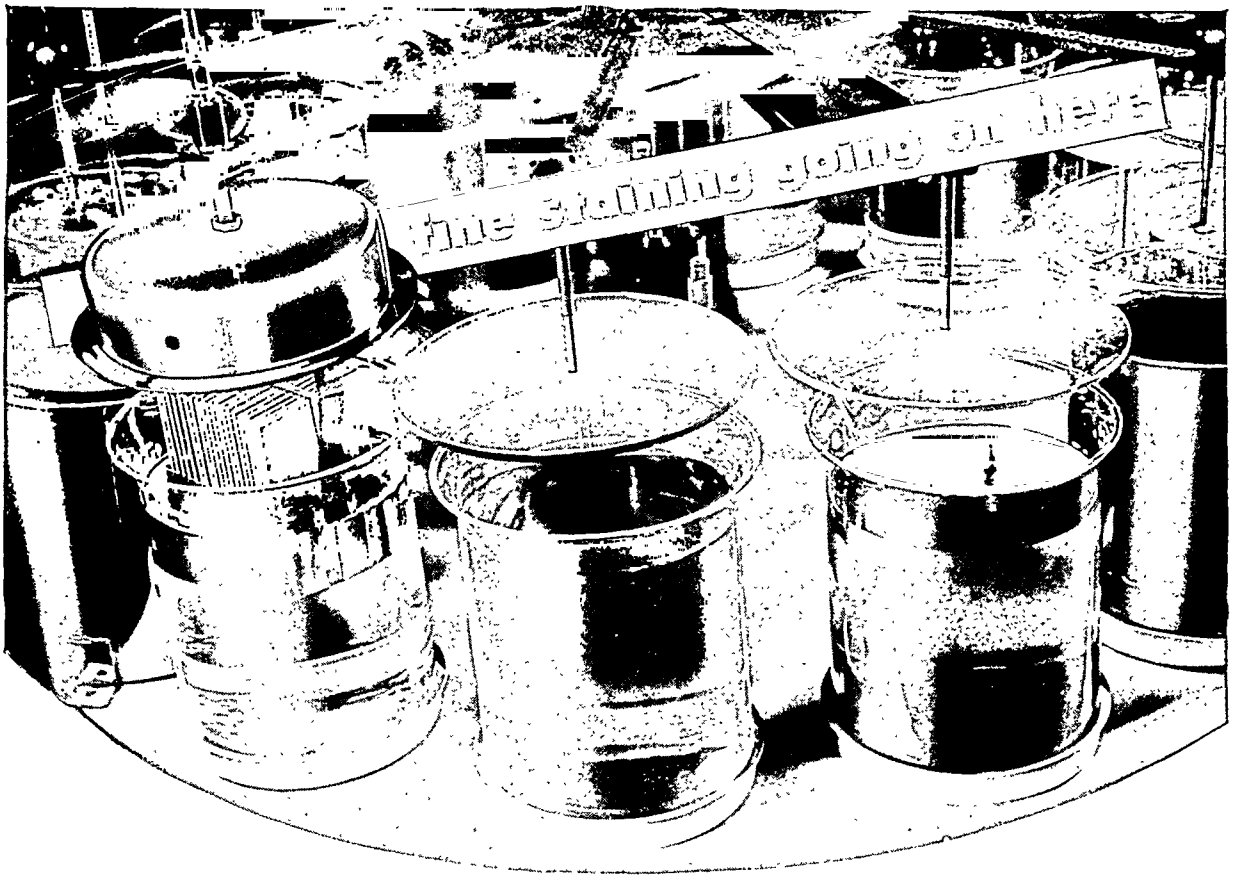
- Brucella Abortus
- Brucella Melitensis
- Paratyphoid A
- Paratyphoid B
- Tularense
- Typhoid, H and O
- Klebsiella pneumonia, Friedlander bacillus

a. s. aloe company *Serving Science Since 1860*

1831 Olive St · St Louis, Mo.

aloe

(In writing to advertisers, please mention the Journal—it helps.)



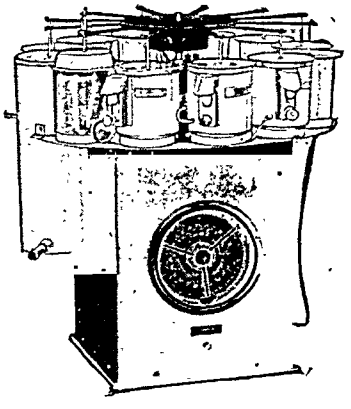
This is a close-up view of a rack of microslides undergoing staining on the **Autotechnicon.** The process is *completely automatic,* and geared to split-second timing. The slides are now descending into one staining-fluid beaker: at the precise moment when the required immersion period has been completed, they will be promptly raised, and rapidly shifted to the next station in the sequence . . . automatically, infallibly. During immersion the slides are rotated gently within the fluid, thus assuring rapid, even stain permeation.

Any technique prescribed by the pathologist may be employed. Once the master dial has been set for the correct time intervals, the method may be repeated time after time, without the minutest variation, and independent of any supervision. Each batch of slides put through is stained in *exactly* the same way, resulting in unvarying uniformity.

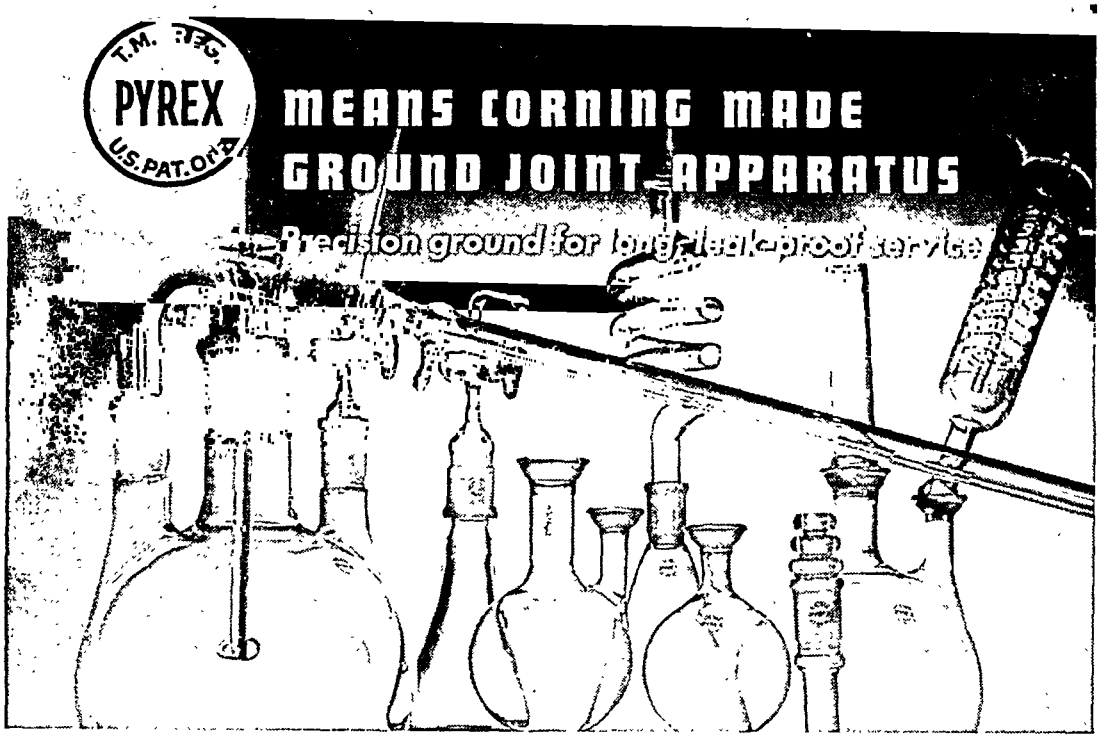
Autotechnicon staining is not only convenient, dependable, time-saving...it produces "Sunday-best" slides . . . *every day.*

Autotechnicon

automatic tissue processing...fixation, dehydration, infiltration, washing, staining



THE TECHNICON COMPANY
215 East 149th Street • New York 51, N. Y.



GENUINE Corning made ⅋ interchangeable ground joint apparatus is identified with the familiar circular PYREX trade mark. This is your guarantee that it is precision ground by the skilled hands that produce all PYREX brand glasses . . . your assurance of economical service.

The secret behind the unvarying quality of PYREX brand ⅋ ware stems from Corning research. The development of abrasives and grinding techniques assures satin surface joints which fit snugly. The unretouched photomicrographs (right) tell the story.

Available now from your laboratory supply dealer who is prepared to handle your glass requirements as well as other laboratory equipment and chemicals from stock.

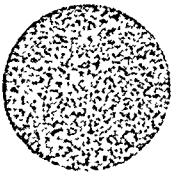
SEE FOR YOURSELF



PRECISION GRINDING —Unretouched photomicrograph (96 magnifications) showing the edge of the ground surface of PYREX brand (⅋) ground joints.



ORDINARY GRINDING —Unretouched photomicrograph (96 magnifications) showing the ground surface on ordinary ground joints.



PRECISION GRINDING —Unretouched photomicrograph (96 magnifications) showing the ground surface on PYREX brand (⅋) ground joints.



ORDINARY GRINDING —Unretouched photomicrograph (96 magnifications) showing the edge of the ground surface on ordinary ground joints.

Stocked by Leading Laboratory Supply Houses

CORNING means research in GLASS

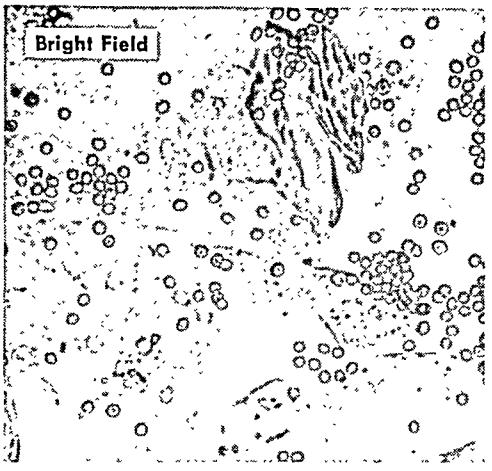
T.M. REG. PYREX U.S. PAT. OFF.

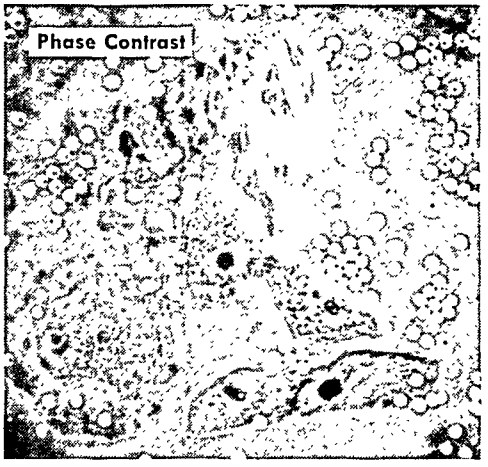
CORNING GLASS WORKS • CORNING, N. Y.

LABORATORY GLASSWARE

TECHNICAL PRODUCTS DIVISION: LABORATORY GLASSWARE • SIGNALWARE • GLASS PIPE • GAUGE GLASSES • LIGHTINGWARE • OPTICAL GLASS • GLASS COMPONENTS

• *Sediment from centrifuged specimen of urine. Phase contrast more clearly differentiates between types of epithelium and casts, and shows presence of pus cells and bacteria.*





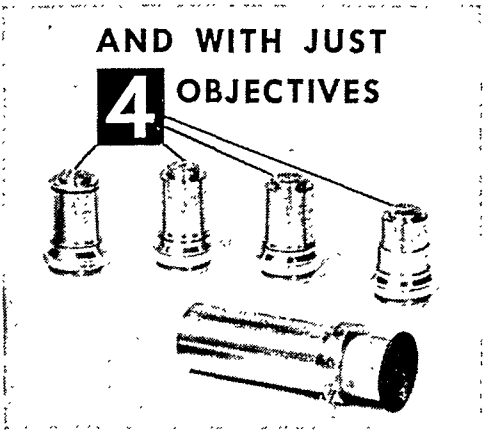
Here's TOP IMAGE CONTRAST

with ANY thin transparent specimen

TOP image contrast with any thin *transparent specimen* is obtained by means of the ¼ wave length annulus. This is conclusively verified by findings of recognized authorities, *plus* the theory of diffraction gratings, *plus* actual interferometer tests. Maintaining this critically precise coating is possible only by B&L high mechanical and optical precision.

Best results are also obtained by positive *contrast image* (denser substances are seen as darker tones), and *balanced intensity of direct and diffracted light beams.* This is the result of B&L accurately controlled coating processes. Of course, you want *speed, simplicity, and ease of operation.* You have it with B&L simplified accessories.

There are just four objectives, a

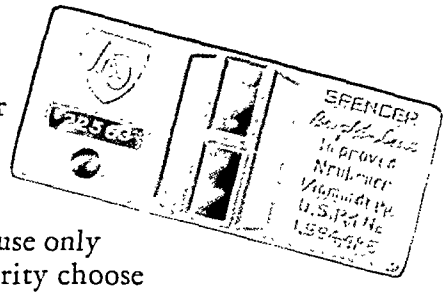


condenser assembly, auxiliary telescope and filter. It takes only a few minutes to put into operation, and is adaptable to most standard microscopes equipped with a substage rack and pinion.

Write for demonstration, and literature to Bausch & Lomb Optical Co., 652-F St. Paul St., Rochester 2, N. Y.

BAUSCH & LOMB *Simplified* PHASE CONTRAST ACCESSORIES

Only an **ACCURATE** Blood Count is Worth Taking



To aid in correct diagnosis and proper treatment of patients, a blood count *must* be accurate. That is why most technicians follow accepted techniques with utmost care . . . why they use only the finest equipment . . . why the majority choose the famous Spencer "Bright-Line" Haemacytometer.

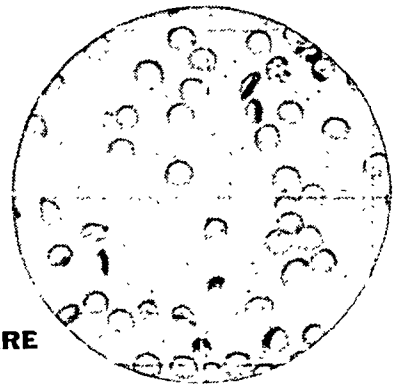
Only **BRIGHT-LINE Chambers offer these 6 Safeguards to Accuracy**

GREATER CONTRAST BETWEEN CELLS AND BACKGROUND

Cells appear dark, lines bright, background gray. Each cell is easily counted—even those which overlap lines.

MORE EVEN DISTRIBUTION OF CELLS

Surface tension differences between metalized surface of chamber and glass surface of cover cause cells to distribute more evenly and chamber to fill more readily.



SIMULTANEOUS RESOLUTION OF BLOOD CELLS AND RULINGS

It is not necessary to stop down the condenser of your microscope since blood cells and rulings can be clearly resolved at full diaphragm opening.

METALIZED BACKGROUND ELIMINATES GLARE

Absence of glare provides clearer vision and reduces eyestrain.

INDENTATION OF UNDERSIDE PROLONGS USEFUL LIFE

Introduction of a slight concavity directly under rulings prevents scratches appearing on lower surface of chamber. Such scratches impair counting accuracy.

PRODUCTION TOLERANCES ARE HELD TO THOSE RECOMMENDED AND SET FORTH BY THE U. S. BUREAU OF STANDARDS

American Optical COMPANY
Scientific Instrument Division
Buffalo 15, New York

When you buy, specify "Bright-Line" and accept no substitutes. Immediate delivery from your supplier or write Dept. T16.

Makers of Medical Optical Instruments for Over 100 Years

SPENCER

THE NEW SPENCER PHASE MICROSCOPE



10960

for Study of Living Organisms and Other Transparent Materials of Inherently Low Contrast

The Phase Microscope is a valuable tool for many specific applications not possible with other models. It complements rather than replaces existing methods of microscopy.

The Spencer No. 18MLSP phase microscope with combination binocular and monocular body, phase turret condenser and quadruple revolving nosepiece with four phase objectives, is ideal where a wide variety of phase contrast studies are being made.

The Spencer phase turret condenser is recommended where rapid changes from high to low powers are frequently required. It has a condenser of the Abbe type with two lens elements and an iris diaphragm supplied in a centerable mount. The rotable turret has four individually centerable cells with interchangeable annular diaphragms and one clear aperture.

These diaphragms may be centered conveniently by use of the centering telescope and a pair of special wrenches supplied with the condenser, and can be parcentered individually and conveniently to any series of four objectives in the nosepiece so that no further adjustments are needed when changing from one magnification to another.

10960—MICROSCOPE, PHASE. Spencer 18MLSP combination binocular and monocular with quadruple nosepiece; built-on ungraduated, mechanical stage; 16, 8, 4 mm dry and 1.8 mm oil immersion dark contrast-medium objectives; centering telescope; paired 10X Huygenian eyepieces; turret condenser, N.A. 1.25 with 4 annular diaphragms and 1 clear aperture; complete with diaphragm centering wrenches and in leatherette covered hardwood cabinet with drawer to hold phase turret condenser..... 965.00

Further Details on This and Other Models and Recommendations on Proper Phase Objectives for Specific Applications will be sent on Request.

SERVING SCIENCE THROUGH SUPPLY

Write, teletypewrite, wire or phone Dept. JCP-6 our nearest office-warehouse:

WILL CORPORATION
LABORATORY APPARATUS AND CHEMICALS
ROCHESTER 3, N. Y. • NEW YORK 12, N. Y.
SOUTHERN SCIENTIFIC CO., INC., ATLANTA 3, GA.
BUFFALO APPARATUS CORP., BUFFALO 5, N. Y.

FIVE

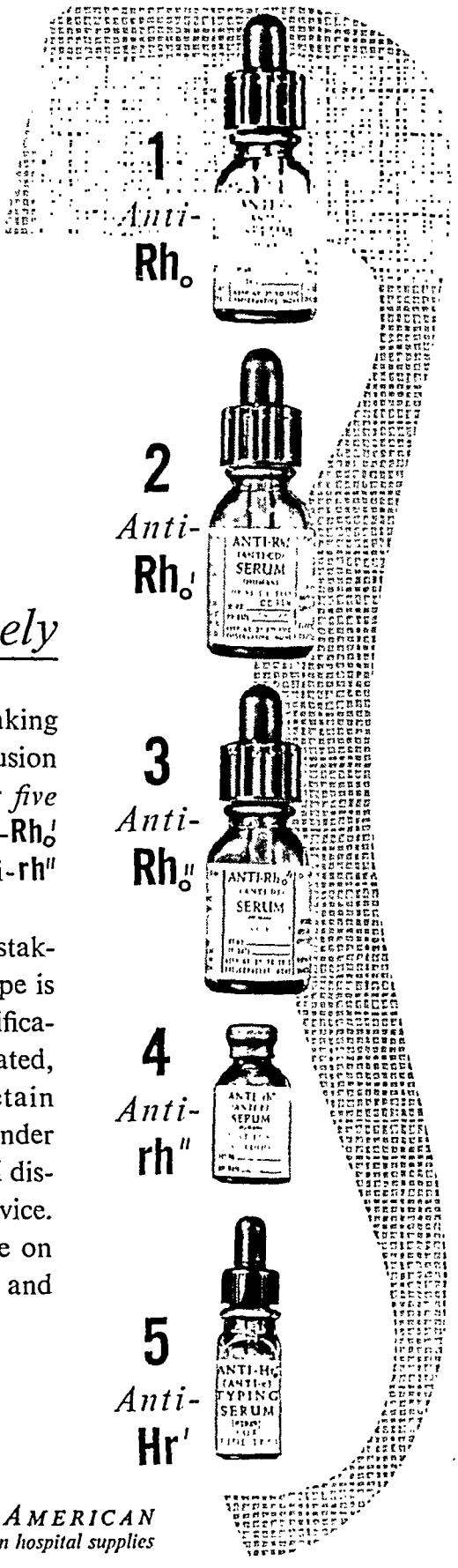
Anti-(Rh) Serums

to test every blood—completely

More and more pathologists are making *complete* Rh determinations for blood infusion cases. They're turning to American for *five* potent serums—Anti-Rh_o (Anti-D), Anti-Rh_o^I (Anti-CD), Anti-Rh_o^{II} (Anti-DE), Anti-rh^{II} (Anti-E) and Anti-Hr^I (Anti-c).

The slide method gives accurate, unmistakable readings in two minutes. No microscope is needed. Serums, prepared to N. I. H. specifications, may be used with fresh, clotted, oxalated, heparinized or citrated blood. They retain original potency over a long period even under adverse conditions. Six strategically located distributing centers assure you of prompt service.

WRITE today for descriptive literature on AMERICAN'S complete line of blood-typing and blood-grouping serums.





PLAN WITH AMERICAN
... the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)



Expect **Accuracy**... not *Alchemy*

with the ROUY **PHOTROMETER** by *Leitz*

The Rouy Photrometer is pre-calibrated for 38 clinical tests to meet the most commonly required needs in clinical analysis—not to set a record in the number of tests claimed possible. It can, however, be readily calibrated for *any test scientifically suited to colorimetric technique.*

Advanced and unique improvements make possible the Photrometer's greater accuracy, ideal simplicity. Ten narrow spectral band filters cover the entire visible spectrum. Pre-calibration and an Individual Handbook eliminate all need for calculation or preparing standards*.

Leitz Rouy Photrometers and Colorimeters are now serving more than 8,300 satisfied users. Each instrument is made to exacting standards of precision and sturdiness by Leitz, first large-scale producer of photoelectric colorimeters.

Pre-calibrated for 38 tests—$257.80 **Pre-calibrated for 22 tests—$182.80**

**Accurate reagents available through Leitz Solution Supply Service.*

For information, write

E. LEITZ, Inc., 304 Hudson St., New York 13, N.Y.

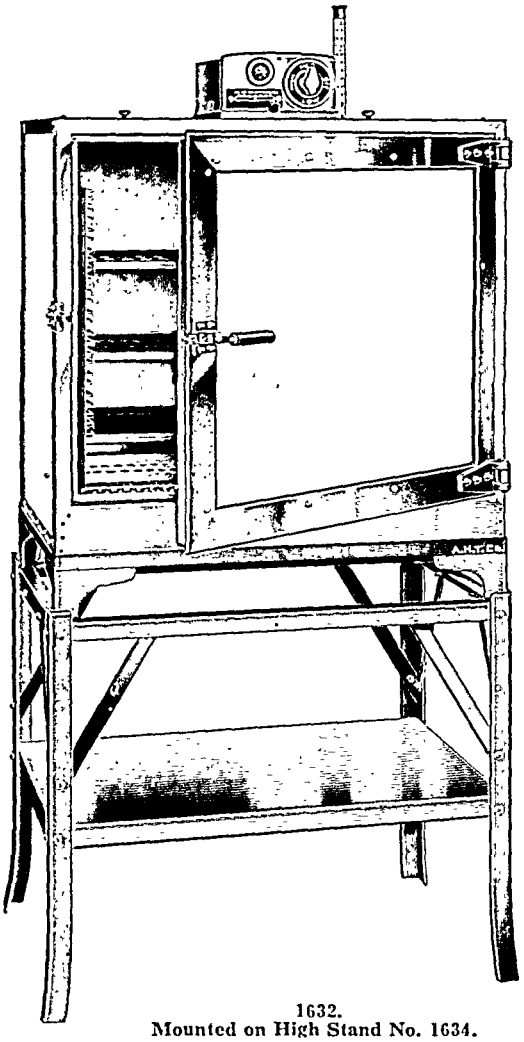
LEITZ SCIENTIFIC INSTRUMENTS • MICROSCOPES • BINOCULARS • LEICA CAMERAS AND ACCESSORIES

(In writing to advertisers, please mention the Journal—it helps.)

WEBER ELECTRIC

HOT AIR STERILIZERS

IN OUR STOCK FOR IMMEDIATE SHIPMENT



1632.
Mounted on High Stand No. 1634.

HOT AIR STERILIZER, Weber Electric. With automatic control and pointer scale for approximate setting at any desired temperature between 150° and 180°C. Constructed of asbestos transite, 1-inch thick, and Monel metal, so that all possibility of rusting or corrosion is avoided.

Shelf brackets 1½ inches apart provide for a variety of shelf placement, and shelves can be loaded while partially withdrawn. Dimensions are designed for convenient and economical handling of pipettes in boxes, Petri dishes and flasks of the sizes most generally used.

Current consumption at 115 volts is 17.5 amperes for operation at 170°C for 1630, and 25.5 amperes for 1632. Time required for reaching this temperature from ordinary room temperature, with sterilizing chamber empty, is approx. 1¾ hours for 1630, and 2 hrs. for 1632.

Operating sensitivity, i.e., maximum variation in temperature at thermometer bulb, when operating empty at a constant temperature of approx. 170°C, is ±1.0°C.

Operating uniformity, i.e., maximum variation in temperature throughout working shelf space, when operating under above conditions, is ±8.0°C.

1630. Hot Air Sterilizer, Weber Electric, as above described, with Monel metal finish, inside dimensions 15 inches high × 18 inches wide × 18 inches deep. With two shelves, thermometer 200°C, and complete operating directions. On low stand, i.e. angle iron feet 3½ inches high. Power consumption 2000 watts. For 115 volts, a.c.....**361.50**

1632. Ditto, but with inside dimensions 23 inches high × 26 inches wide × 18 inches deep. On low stand, i.e. angle iron feet 3¼ inches high. Power consumption 3000 watts. For 115 volts, a.c.....**542.00**

1633. High Stand, only for 1630; of angle iron, aluminum finish, 26½ inches high**23.30**

1634. Ditto, for 1632; 26½ inches high**29.65**

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

CABLE ADDRESS, "BALANCE", PHILADELPHIA

EXPERIMENTAL OVARIAN TUMORS IN RATS*

GERSON R. BISKIND, M.D. (SAN FRANCISCO), AND MORTON S. BISKIND, M.D. (NEW YORK)

From the Departments of Pathology, Mount Zion Hospital and the University of California Medical School, San Francisco, California

The observation of Golden and Sevringhaus[12] that an ovary transplanted into the mesentery of a castrate female rat was viable but could not induce estrogenic stimulation of the vagina indicated that the hormones it elaborated were destroyed by the liver. Some of our previous experiments confirmed this finding[3] and, by using a modification of this technic, we showed that the estrogen-inactivating function of the liver could be impaired by altering the nutritional state of the animal.[5, 6, 8] The experiments to be described are based on the ability of the liver to inactivate the estrogen elaborated by an ovary transplanted to the spleen, and they demonstrate the progressive changes that take place in the transplanted ovary, with the eventual formation of a neoplasm.

Since our first description of the induction of this type of ovarian tumor[7] and our report of additional findings,[4] these specific experimental conditions have been duplicated by others and the growth of intrasplenic ovarian transplants has been confirmed. Furth,[10] Furth and Sobel,[11] and Li and Gardner[18] have described typical granulosa cell tumors in intrasplenic transplants in mice. Li[17] found the same changes after intrasplenic transplantation of an ovary into a castrate male mouse. Li and Gardner[19] obtained granulosa cell tumors after intrapancreatic ovarian grafts. Lipschütz, Ponce de Leon, Waywood, and Gay[21] and Lipschütz[20] have mentioned luteal proliferation in intrasplenic ovarian transplants in guinea pigs. The production of this type of growth in rabbits has been obtained by Peckham, Greene, and Jeffries.[22] Heller and Jungck[13] described the growth of the transplant, and the tumors have been observed in rats by Peckham, Greene, and Jeffries.[22]

MATERIALS AND METHODS

Most of the rats used in this study were of the Long-Evans strain; some animals of the Sherman strain and a hooded strain of uncertain lineage were also employed. Immature, young adult, and old animals were included. The animals were oophorectomized either through a bilateral lumbar approach, or through a midline abdominal incision. One ovary was cleared of the adherent fat and oviduct, and either all or a large part of it was inserted in a pocket under the capsule of the spleen. This procedure was performed at the time of castration. In one group of control animals, the ovary was placed in the kidney. The animals were maintained under optimum conditions on a complete stock diet of known composition. Food and water were available at all times. The vaginal

* This investigation was supported in part by a research grant from the National Cancer Institute of the National Institute of Health, U. S. Public Health Service.

Received for publication, February 17, 1949.

smears were examined at fairly regular intervals. Most of the animals were sacrificed by exsanguination after ether anesthesia, the remainder by a lethal dose of pentobarbital sodium. Blocks of the transplant were immediately fixed in Bouin's solution, Zenker's solution or 10 per cent formalin. The other organs were usually placed in Bouin's solution, except the hypophysis which was fixed in Zenker's solution. The blocks of tissue were dehydrated, embedded in paraffin, sectioned and stained in the usual manner. The routine stains were hematoxylin and eosin, a silver stain for reticulum, and Masson's connective tissue stain.

OBSERVATIONS

To determine the effect of transplantation of the ovary into the spleen, animals were sacrificed at specific intervals after the operative procedure. The following composite descriptions are based on the examination of tissues obtained from the animals listed in Tables 1, 2 and 3. Presented in these tables are the number of animals used, the age at castration and transplantation and the age of the transplant. A brief note describes the major histologic features of the transplant. The condition of the uterus or vagina is indicated, as is the presence or absence of adhesions around the operative site in the spleen.

The rats listed in Tables 1 and 2 have been classified into groups, according to the presence or absence of adhesions that might possibly permit blood from the spleen to bypass the liver. Data on 92 are listed in Table 1; 12 of these animals had some thin adhesions between the spleen and the parietal peritoneum, and 24 had adhesions between the spleen and the pancreas, omentum, or mesentery. In Table 2 are listed data on 21 animals with prominent adhesions between the spleen and the parietal peritoneum. Thirty-nine of the animals listed in Table 1 were under 40 days of age when operated upon and were classified as immature; most of these animals were included in the group examined after the tumor had grown for about two years. Table 2 includes only 6 animals that lived about one year or longer, and these were 24 to 48 days old at the time of transplantation.

When small, the exact size of the transplant could not be measured too accurately because of the thin capsule and adherent splenic or renal tissue. At this stage, the transplant in the block of tissue was cut in its greatest diameter and when a single measurement is listed in the tables, it represents the greatest diameter taken from this cross section. As the transplant enlarged, it would sometimes protrude to a great extent from the spleen, and more accurate measurements became possible. There is some correlation between the size of the transplant and its age. However, several transplants over 200 days of age remained small, for which there is no apparent explanation. The transplants with vascular adhesions were uniformly smaller than the transplants without adhesions, and none of the former ever grew to the huge size occasionally encountered in the latter. At autopsy, each animal was examined carefully for evidence of metastasis. The liver and lungs were cut into thin slices. There was no instance of metastasis in any of the animals.

Some of the transplants were cystic, and these cysts were lined either by cor-

TABLE 1

TRANSPLANTATION OF OVARY TO THE SPLEEN OF CASTRATE FEMALE RATS*

TISSUE NO.	AGE AT		TRANSPLANT		PREDOMINANT TYPE OF VAGINAL SMEAR†	ADHESIONS††	UTERUS OR VAGINA§	PATHOLOGIC FINDINGS§§
	Castration and Transplantation	Autopsy	Age	Size				
	days	*days*	*days*	*mm.*				
2764	75	75	⅓			0		Early nec. of tr. Acute inflam.
2765	80	81	1			0		Nec. of tr. Acute inflam.
1139	175	176	1			0		Nec. of tr. Acute inflam.
2766	30	31	1			0		Nec. of tr. Acute inflam.
2690	99	100	1			0		Nec. of tr. Acute inflam.
1145	175	177	2			0		Few actively growing foll.
2736	61	63	2			0		Nec. of original c.l. and ov. tissue
2770	76	78	2			0		Nec. of original c.l. and ov. tissue
2774	76	79	3		D	0		Actively growing foll. at periphery, nec. and inflam. in center
2696	99	103	4		D	0		Actively growing foll. at periphery and small c. l.
1150	175	180	5		E	0		Foll. at periphery. No c.l.
2542	46	52	6		D	0	a	Newly developing foll. Small c.l.
1015	127	134	7		E	0		New foll. at periphery, some cystic. Large and small c.l.
2787	81	89	8	4	D	0		Inflam. subsiding with fibrosis. Many developing foll.
2746	68	78	10	5	D	0	a	Numerous prim. foll. Several cystic c.l.

* The animals listed in this table had both ovaries removed, and either the whole or a half of the left ovary inserted into the spleen. The age of the animals at the time of operation, and the age of the animals at autopsy are indicated. The transplant did not lend itself to accurate measurement unless it was very large; where one diameter was listed it was obtained by measuring the cross section of the tumor in its greatest diameter.

† D, diestrous; E, estrous

†† O, no adhesions; +, adhesions to pancreas, omentum or mesentery; ++, adhesions to abdominal wall, kidney or uterine horn

§ a, atrophy; es, estrogenic stimulation; ES, strong estrogenic stimulation

§§ c.l., corpora lutea; fib., fibrous; foll, follicles; gran., granulosa; hemorr. infarc., hemorrhagic infarction; inflam., inflammation; lut., luteal; nec., necrosis; ov., ovarian; p.m., postmortem; prim., primordial; pseudotub., pseudotubular; str., structures; tr., transplant; tub., tubular

TABLE 1—*Continued*

TISSUE NO.	AGE AT		TRANSPLANT		PREDOMINANT TYPE OF VAGINAL SMEAR†	ADHESIONS††	UTERUS OR VAGINA§	PATHOLOGIC FINDINGS§§
	Castration and Transplantation	Autopsy	Age	Size				
	days	*days*	*days*	*mm.*				
2384	28	39	11	4	D	+	a	Many large c.l., cystic or fib. centers
2648	84	97	13	2	D	0	a	Some cystic foll. Several c.l.
2753	61	76	15	4	D	0	a	Active foll. and c.l.
2572	46	62	16	4	D	0	a	Many c.l. and foll.
2782	26	43	17	8	D	++	a	Many c.l. and foll.
2720	99	119	20	5	D	0	a	Many c.l. and foll.
2769	61	82	21	5	D	0	a	Many c.l., some with central fib. core. Many foll.
2667	84	110	26	5	D	0	a	Many c.l., some cystic. Some cystic foll.
1196	175	201	26	8	D	0	a	Many c.l., some cystic. Active foll.
2792	61	91	30	7	D	0	a	Many c.l. and foll.
1535	194	224	30	5x5	D	+, ++	a	Many c.l., some with central fib. core
2786	34	68	34	7	D	+	a	Many c.l.; central fib. core rarely present
2848	77	116	39	4x5	D	0	a	About 10 c.l. Prominent fib. stroma. Few foll.
1536	195	240	45	6x5x4	D	0	a	Many distinct c.l., some slightly cystic
2522	25	88	63	5x5	D	+	a	C.l., some cystic, others small
2707	29	92	63	10	D	0	a	Multiple c.l. Some poorly defined scattered foll.
2756	91	164	73	6	D	0	a	Many large c.l., some with central fib. core
2042	33	130	97	8	D	0	a	Large uniform c.l.
2162	37	146	109	2	D	+	a	Large c.l., poorly outlined
2046	46	175	129	10	D	0	a	Some variation in size of c.l.
1205	68	209	141	8	D	+	a	Many c.l., poorly outlined, some with central fib. core; cyst in center. Few foll.
2700	25	175	150	10x10x10	D	+	a	Compactly placed, indistinctly outlined c.l. Few foll.
1182	30	180	150	15x8x8	D	0	cs	Cystic tr., many c.l. Few foll.

TABLE 1—*Continued*

TISSUE NO.	AGE AT		TRANSPLANT		PREDOMINANT TYPE OF VAGINAL SMEAR†	ADHESIONS††	UTERUS OR VAGINA§	PATHOLOGIC FINDINGS§§
	Castration and Transplantation	Autopsy	Age	Size				
	days	*days*	*days*	*mm.*				
2699	25	175	150	15	D	+	a	Cystic tr., many c.l. Few foll.
2713	25	176	151	9	D	0	a	C.l. poorly outlined. Some foll.
1270	22	175	153	9	D	+	a	Tr. cystic, c.l. in wall. No foll.
1271	68	223	155	11	D	+	a	Cyst at one end, c.l. compressed
2242	38	195	157	10	D	+	a	Luteoma
1280	24	183	159	8	D	0	a	C.l. variable size and shape. No developing foll.
2127	47	220	173	10	D	0	es	Luteoma
2703	28	203	175	20x20x20	D	+	es	Luteoma
2784	27	214	187	9	D	0	a	Many distinct c.l. No foll., one unencapsulated zone 2–3 x size of c.l. Early luteoma
2737	24	224	200	9	D	0	a	Luteoma
2321	45	270	225	5		++	a	Many small c.l. with cellular nodule typical of luteoma
2254	33	260	227	14x9	D	+		Luteoma with questionable gran. cell tumor
2322	45	278	233	4	D	0	a	Poorly defined c.l. No foll. No luteoma
2267	32	275	243	9	D	+	a	Cystic tumor, c.l. lining cyst. Rare foll.
2527	180	446	266	7	D	0	a	Small luteoma with atypical cell nests
2793	73	348	275	11	D	0	a	Small luteoma
1207	62	371	309	6	D	0	a	Coalesced c.l. Luteoma not definite.
38**S	4 mo.	437	317	15x13x10	D	0		Luteoma with gran. cell tumor
25**S	6 mo.	499	319	5x4x3	E	++	es	Luteoma
14**S	6 mo.	505	325	8x7x7	E	++	es	Luteoma
2342	48	380	332	60x50x50	D	0	a	Luteoma
2484	31	365	334	13	E	++	es	Gran. cell tumor in luteoma
15**S	6 mo.	517	337	7x5x5	E	++	es	Luteoma
34**S	4 mo.	466	346	8x9x5	D	0	a	Luteoma

** S, Sherman rat

TABLE 1—*Continued*

TISSUE NO.	AGE AT		TRANSPLANT		PREDOMINANT TYPE OF VAGINAL SMEAR†	ADHESIONS††	UTERUS OR VAGINA§	PATHOLOGIC FINDINGS§§
	Castration and Transplantation	Autopsy	Age	Size				
	days	*days*	*days*	*mm.*				
35**S	4 mo.	466	346	10x11x12	E	0	ES	Luteoma with gran. cell tumor
2354	47	396	349	10x8x8	E	+	a	Luteoma
46**S	6 mo.	530	350	15x10x10	E	+, ++	ES	Luteoma with gran. cell tumor
48**S	6 mo.	530	350	12x7x7	E	0	a	Luteoma
1321	28	393	365	35x20x17	E	+	es	Luteoma with gran. cell tumor
2584	38	414	376	1.3x1x0.9	E	+	es	Luteoma with gran. cell tumor
10**S	184	579	395	10x10x12	D	0	es	Luteoma
2711	30	431	401	17x10	E	0	es	Gran. cell tumor in luteoma
2653	38	454	416	1.8x1.3x1.4	E	0	es	Gran. cell tumor in luteoma
1369	28	447	419	40x30x25	E	+	es	Luteoma; hemorr. infarc. from twisted pedicle
2708	31	482	451	28x25x20	E	0	es	Gran. cell tumor in luteoma
2709	31	482	451	16x15x15	E	0	es	Luteoma with gran. cell tumor
2676	33	521	488	40x30x30	D	0	a	Luteoma, cystic
2974	38	550	512	18	D	0	ES	Cystic gran. cell tumor
1518	94	643	549	30x25x23	E	+	a	Luteoma
2794	30	580	550	14x8x7	E	++	es	Multilocular cyst, luteoma
2998	38	603	565	30x30x25	E	0	es	Luteoma and gran. cell tumor
1520	90	715	625	45x40x35	D	+	es	Gran. cell tumor in luteoma
1172	31	662	631	30x20x15	E	+, ++	es	Gran. cell tumor in luteoma
1356	30	674	644	50x50x30	D	+		Hemorr. infarc. and p.m. autolysis of large cellular tumor
1284	31	699	668	11	E	+	es	Luteoma and gran. cell tumor
11**S	184	852	668	40	E	0	ES	Gran. cell tumor in luteoma
13**S	184	897	713	28x22x20	E	0	ES	Gran. cell tumor in luteoma
1385	31	770	739	26	D	0	a	Luteoma with gran. cell pattern

TABLE 1—*Concluded*

TISSUE NO.	AGE AT		TRANSPLANT		PREDOMINANT TYPE OF VAGINAL SMEAR†	ADHESIONS††	UTERUS OR VAGINA§	PATHOLOGIC FINDINGS§§
	Castration and Transplantation	Autopsy	Age	Size				
	days	*days*	*days*	*mm.*				
1505	45	835	790	17x15x14	D	0	a	Luteoma with gran. cell pattern in some areas
1506	45	835	790	55x40x30	E	++	es	Gran. cell tumor in luteoma. Adenofibroma of breast
1509	30	869	839	5x3x3	E	0	es	Luteoma with gran. cell elements
1508	30	886	856	25	E	++	es	Luteoma with gran. cell elements
1507	45	914	869	3x1.5x2	E	+	ES	Luteoma with minute gran. cell elements
1510	30	981	951	70x45x40	E	+	ES	Luteoma with gran. cell elements showing severe nec.

pora lutea or, rarely, by follicular cells. Some of the tumors also were cystic, and the cysts were lined by cells of the tumor, either luteal or granulosa cells. Sometimes the cysts contained clear fluid, occasionally blood. In 3 instances, the spleen and the large tumor growing in it underwent hemorrhagic infarction due to torsion or twisting of the pedicle; this process killed the animals.

In Table 1 are recorded data on 50 animals that were sacrificed after the transplant was 157 days old. Of these, 46 animals had a tumor, either a luteoma or a granulosa cell tumor, or both. The granulosa cell tumor alone was found in only 8 animals. There is no apparent explanation why the transplant, usually enlarged, was not transformed into the tumor in the remaining 4 animals. Only one of these 4 animals had perisplenic adhesions, none showed estrogenic stimulation of the uterus or vagina. Tumors were present in the 36 animals sacrificed 317 days after transplantation. A granulosa cell pattern was apparent as early as 227 days after transplantation, it was definite after 334 days, and from that period on, was present in 23 out of 33 tumors.

On microscopic examination it was found that the immediate effect of transplantation of ovary into the spleen was extensive infarction of the transplanted tissue with an associated acute inflammatory reaction (Fig. 1). The sections reveal that within the first two or three days after transplantation there were variations in the intensity of the inflammatory reaction with evidences of healing. The ovarian parenchyma underwent infarction and disappeared so that only the ghosts of the follicles and corpora lutea could be recognized. The luteal tissue invariably disappeared. Occasionally a rare follicle persisted, particularly among those at the edges of the transplant. The formation of a few primordial

follicles as early as the third or fourth day was evidence that some ovarian tissue had remained viable. From this time on, there was a continuous development of new follicles that appeared quite normal. They appeared initially as simple arrangements of rings of follicular cells around ova. As they grew, the follicular cells increased in number and mitotic activity became intense. A cumulus oophorus was formed and the ovum resided in it. A thin zone of theca interna could be distinguished around each enlarging follicle. Luteinization of the mature follicle apparently took place from the periphery inward, usually after the ovum had undergone dissolution. Hemorrhage in the follicle was not common. Sometimes a follicle remained partially luteinized. Luteinization of the follicle *occurred no sooner than the fifth or sixth day after transplantation* (Fig. 2). From that time on, the follicles went through the process of luteinization with regularity. These corpora lutea occasionally had a small central zone of hemorrhage that organized into a fibrous tissue core. The corpora lutea were composed of large typical luteal cells arranged in radiating cords separated by a fine capillary network. The corpora lutea showed no evidence of involution once they had reached their maximum size. The pattern from about 8 days to 150 days after transplantation was similar in almost all features (Fig. 3). The original inflammatory reaction had disappeared. There was an orderly continuous development of new primordial follicles that progressed through all stages of the developing follicle. The mature follicle could not rupture, and the ovum died in it. Luteinization of the follicle occurred. These corpora lutea showed no evidence of involution but remained apparently viable. There was a continuous increase in the number of corpora lutea; thus the transplant progressively increased in size. As the transplant became larger, the developing follicles were usually found around the periphery, and were sometimes grouped in clusters. In some transplants they were diffusely scattered. The enlarged transplant often protruded from the spleen, covered by the capsule of the spleen (Fig. 4). At all times a thin fibrous band separated the transplant from the splenic parenchyma.

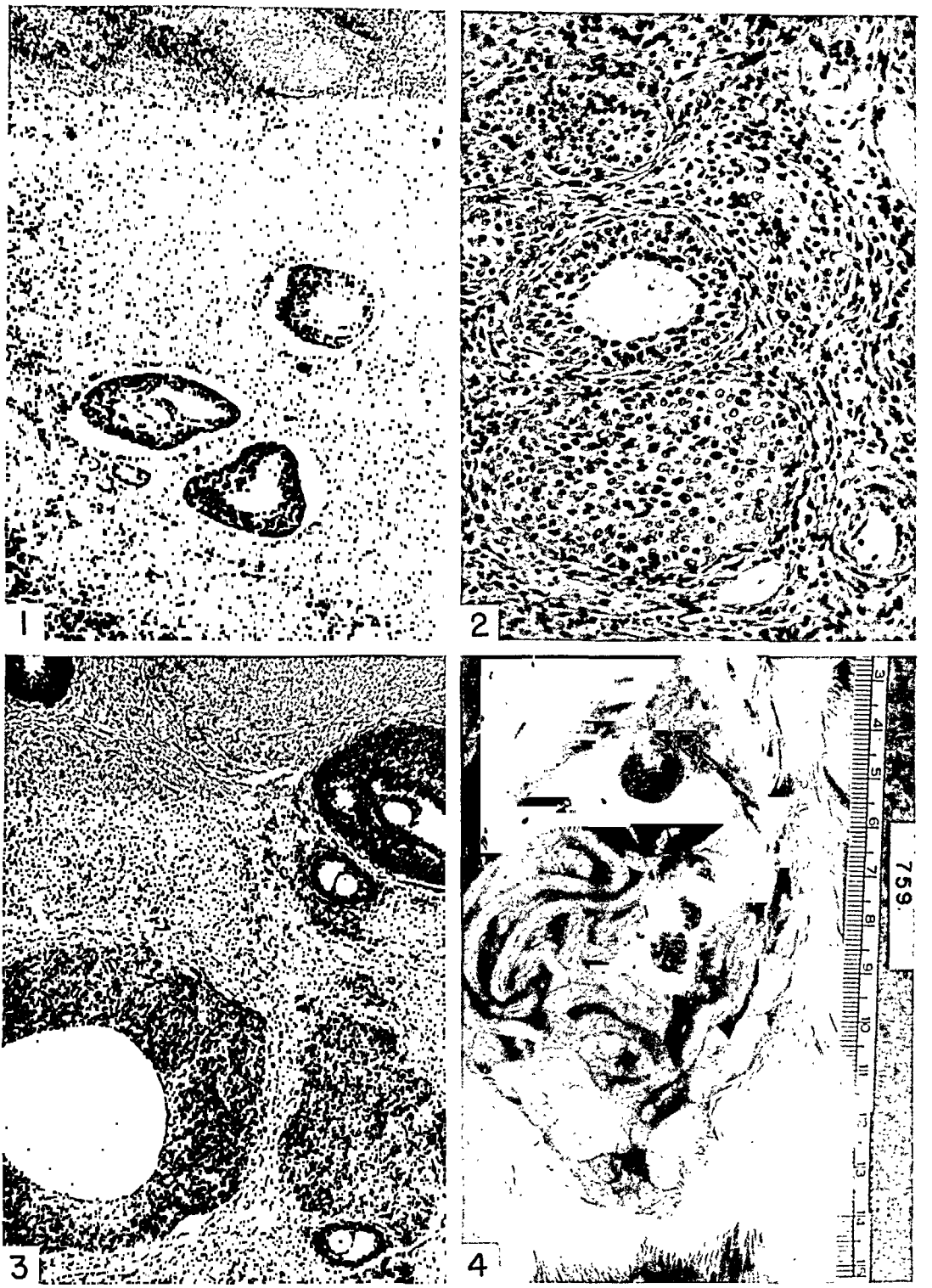
There was a critical period between 150 and 175 days when the transplant first revealed evidence of neoplastic change. The earliest sign of this change was the appearance of a small distinct cellular zone at one point. This nodule was as large as 2 or 3 corpora lutea. The cells in it had lost the usual alignment noted in the average corpus. They were usually compactly arranged in irregularly shaped masses, and often were quite compressed so that only the nuclei were clearly defined. They showed mitotic activity. As this cellular nodule grew, it replaced most of the transplant and pushed aside the remaining corpora lutea.

FIG. 1. (No. 2765-1d.) The ovarian transplant shows three follicles undergoing necrosis. There is severe edema, and an early infiltration of leukocytes and red blood cells. × 30.

FIG. 2. (No. 2542-6d.) Three developing follicles and one luteinized follicle are present. The stroma is edematous and still contains many inflammatory cells. Most of the stromal cells are fibroblasts. × 100.

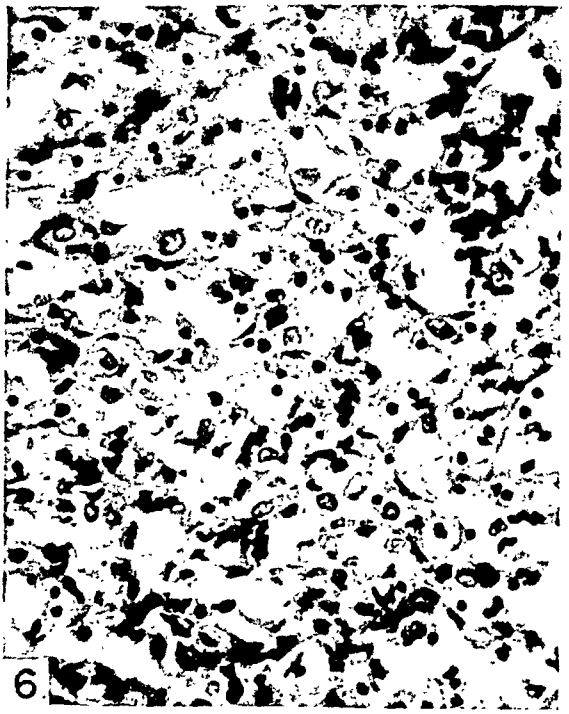
FIG. 3. (No. 2196-66d.) There are numerous developing follicles in all stages and clearly demarcated corpora lutea; the interstitial tissue is scant. × 30.

FIG. 4. (No. 2759-159d.) The transplant is a sharply encapsulated grayish white nodular mass. The individual nodules are about 1 mm. in diameter. Sections showed that each nodule represents a sharply outlined corpus luteum; primordial follicles are extremely rare.



FIGS. 1–4





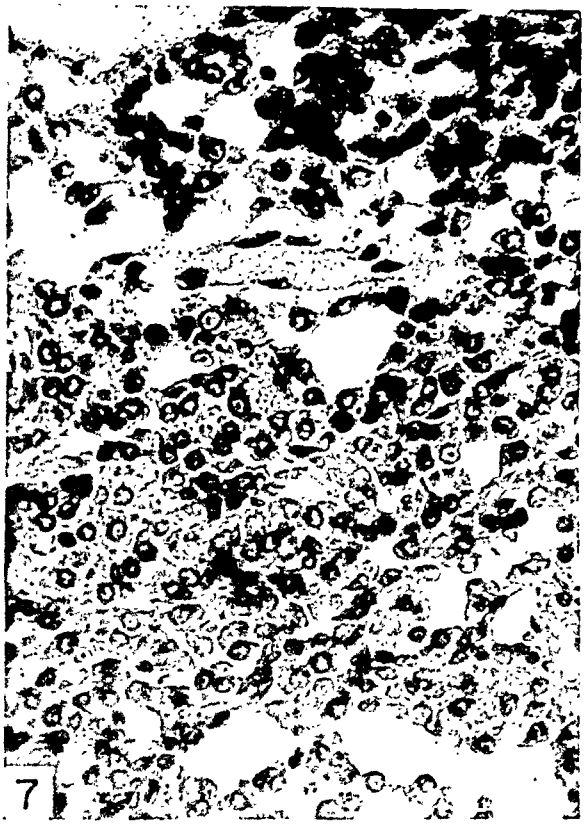




FIG. 5. (No. 2703-175d.) The luteoma is separated from the spleen by a thick fibrous capsule in which there are scattered corpora lutea. × 20.

FIG. 6. (No. 1507-867d.) Granulosa cell tumor. There is an intermingling of small granulosa cells with large vacuolated luteal cells. × 200.

FIG. 7. (No. 2703-175d.) Luteoma. The cells of the luteoma are loosely arranged in some zones. × 200.

FIG. 8. (No. 846-350d.) Granulosa cell tumor. The demarcation between the nests of granulosa cells and the pre-existing luteoma is distinct. The granulosa cell masses have a central cystic zone. × 30.

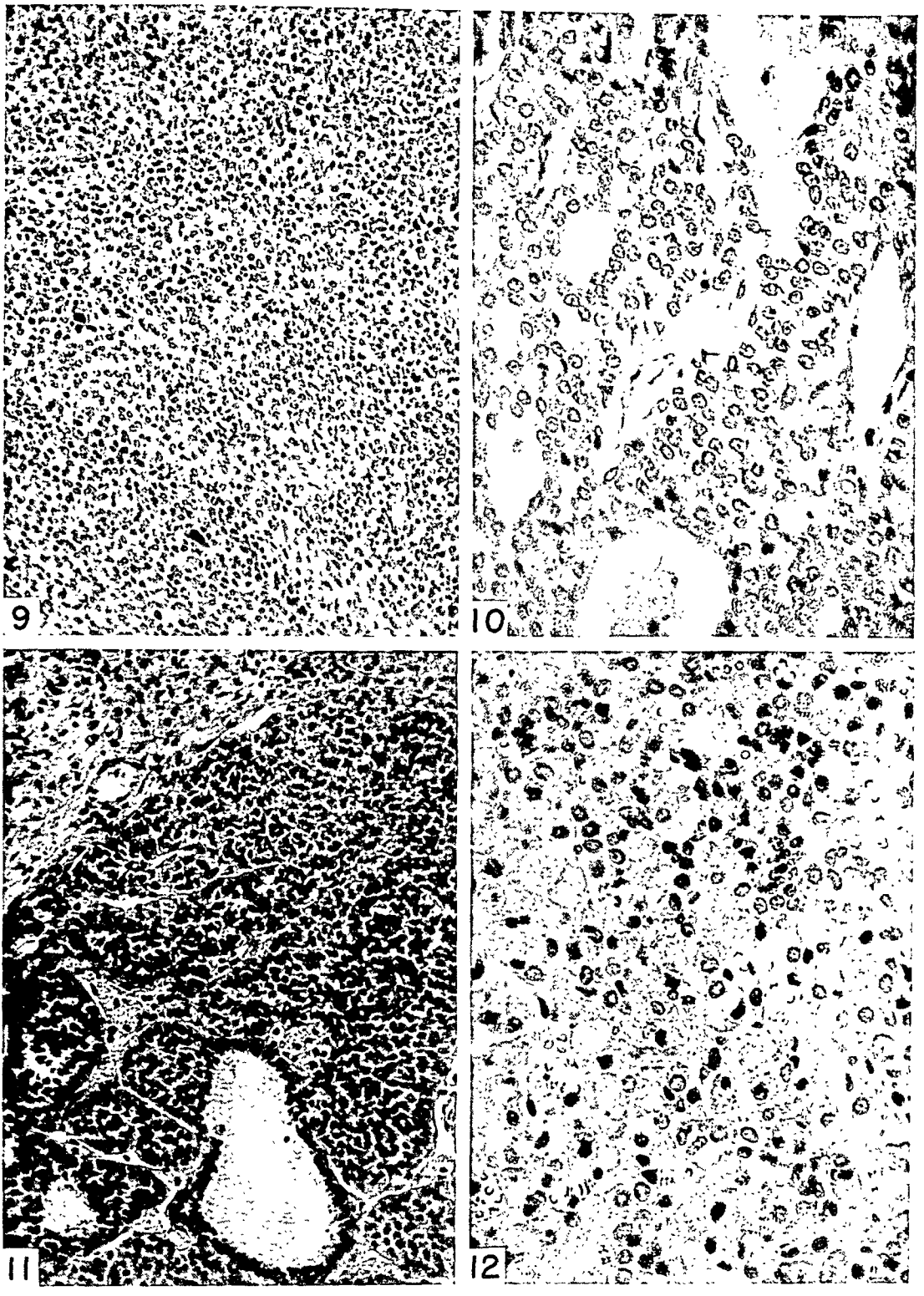


FIG. 9. (No. 1510-951d.) Granulosa cell tumor. The granulosa cells are present as solid sheets of small cells. × 100.

FIG. 10. (No. 1506-790d.) Granulosa cell tumor. The granulosa cells are arranged in thick cords with a formation of small cysts. × 200.

FIG. 11. (No. 2711-401d.) Granulosa cell tumor. The granulosa cells are arranged in small nests, balls, or cysts. Luteal cells are present at one edge. × 100.

FIG. 12. (No. 1271-155d.) The large castrate cells of the anterior lobe of the hypophysis are distinct; basophils and eosinophils are not numerous. × 200.

Usually a distinct fibrous band separated the remaining compressed corpora from the luteoma (Fig. 5). Developing follicles could no longer be found. It was inferred that the luteoma probably arose from a single corpus luteum or a cluster of luteal cells rather than from the coalescence of many corpora lutea; however, it is impossible to be certain of this point at present. The luteoma continued to grow in size and in some instances reached a maximum diameter of 6 or 7 cm. In the larger tumors the pattern remained the same except that the masses of cells in some places were compactly arranged and in other areas were loosely distributed. The cells nearly always retained the luteal appearance (Fig. 6).

After a period of at least 300 days, an additional cellular change took place. Granulosa cells appeared in the luteoma, sometimes as discrete nodules (Fig. 7), other times as diffusely infiltrating nests (Fig. 8). They were much smaller than the luteal cells, and the cell membrane and cytoplasm were not distinct. The nuclei were round and hyperchromatic. Mitotic figures were more numerous. The cells were arranged in sheets, cords, balls, plugs, or small nests, or in a glandular or papillary formation that resembled all common patterns noted in the human type of granulosa cell tumor (Figs. 9 to 11). In some instances the entire luteoma was replaced by these cells. Occasionally, the presence of as many as 4 or 5 different cell types and patterns suggested that the tumor might represent a teratoma composed of undifferentiated cellular elements. These zones were sharply demarcated grossly and varied from gray to white to fleshy pink (Fig. 13).

During the first phase of the development of the transplant, the vagina usually showed a dioestrous smear. Unless adhesions about the spleen were present, it was uncommon to find cells other than leukocytes and a few epithelial cells. If adhesions were present, cornified cells appeared in the smear. In the animals without adhesions, the uterus was usually atrophic. The hypophysis contained many castrate cells in the anterior lobe (Fig. 12). With the appearance of the luteoma, the vaginal smear often showed some evidence of estrogenic stimulation but no clear-cut estrous reaction was observed. Some degree of estrous stimulation often took place in the uterus of these animals. There were several luteomas that showed intense estrogenic stimulation of the vagina, uterus and hypophysis, and it is evident that these tumors formed sufficient estrogen to escape the inactivating process in the liver. In almost every instance, the granulosa cell tumors also produced sufficient estrogen so that some passed through the

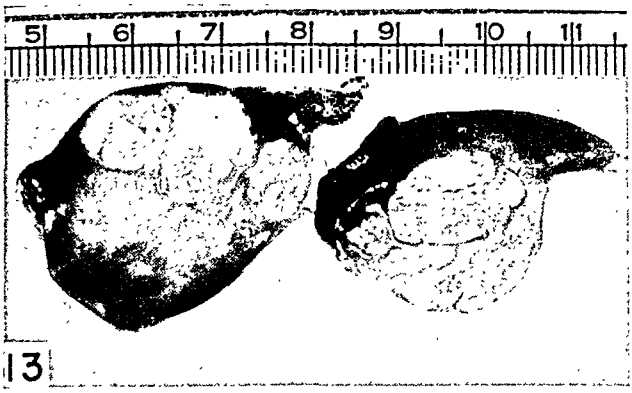
FIG. 13. (No. 1520-625d.) Two sections of a large tumor show that it is composed of several distinct zones that vary from white to pink in color. A portion of spleen can be distinguished on one edge of the tumor.

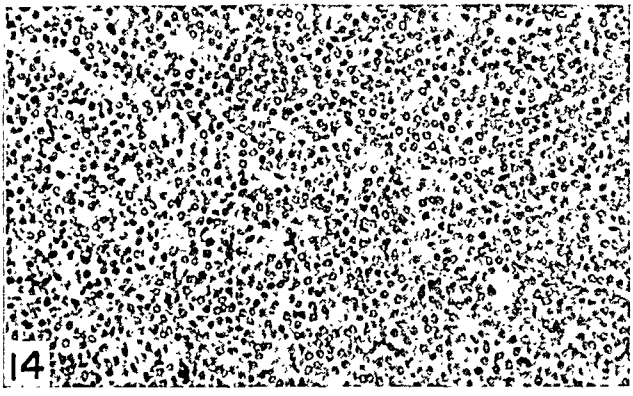
FIG. 14. (No. 1506-790d.) The anterior lobe of the hypophysis, from an animal bearing a granulosa cell tumor, is composed of uniform small chromophobe cells with scant cytoplasm. × 100.

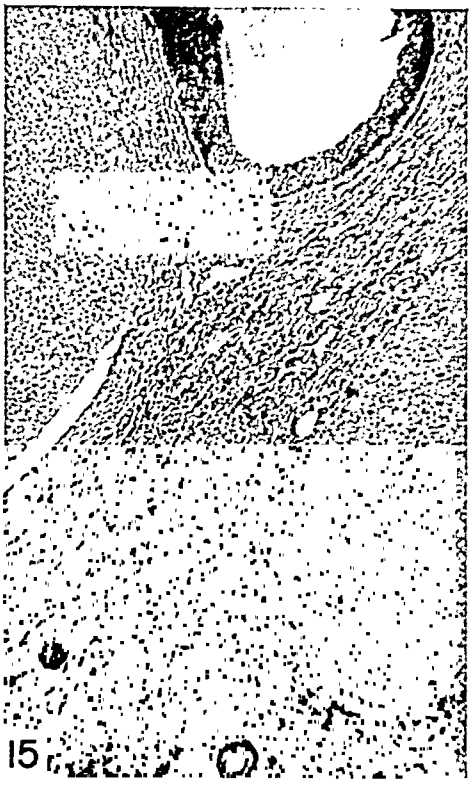
FIG. 15. (No. 2739-201d.) This transplant, from an animal that developed vascular adhesions between the site of the transplant and the abdominal wall, is composed of several small corpora lutea and follicles. A dense fibrous stroma separates these structures. × 30.

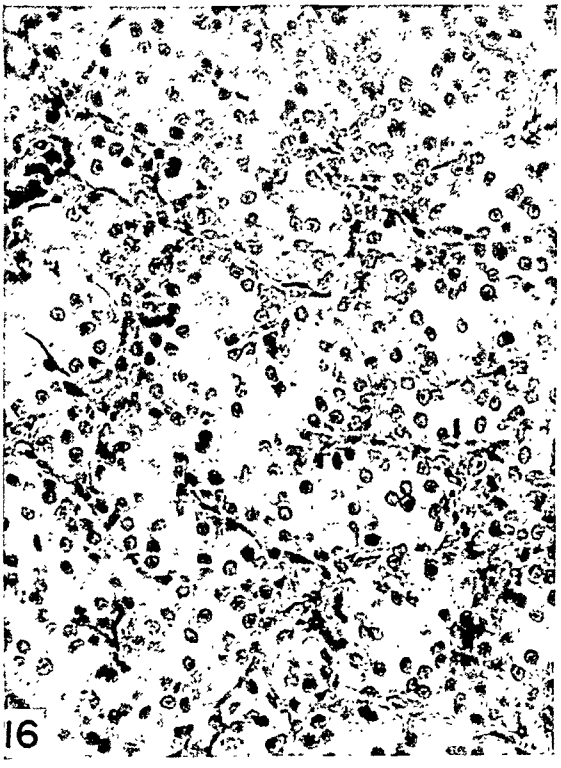
FIG. 16. (No. 1544-605d.) Ovary transplanted into kidney. The transplant shows a uniform pattern of small tubular structures lined by vacuolated cells of the lutein type. This transplant did not contain corpora lutea or follicles. × 200.

FIG. 17. (No. 2810-222d.) Ovary in spleen of hypophysectomized animal. One small and three minute follicles are surrounded by a scant fibrous stroma and represent the entire transplant. × 100.











FIGS. 13–17

TABLE 2

TRANSPLANTATION OF AN OVARY TO THE SPLEEN OF CASTRATE FEMALE RATS* WITH THE FORMATION OF VASCULAR ADHESIONS BETWEEN THE SPLEEN AND THE ABDOMINAL WALL

TISSUE NO.	AGE AT		TRANSPLANT		PREDOMINANT TYPE OF VAGINAL SMEARS**	ADHESIONS†	UTERUS OR VAGINA††	PATHOLOGIC FINDINGS§
	Castration and Transplantation	Autopsy	Age	Size				
	days	*days*	*days*	*mm.*				
2360	24	29	5		E	++		Foll. around periphery. Some cystic c.l.
1014	127	134	7		E	++	es	Some cystic foll. Small c.l.
1169	175	187	12	4	E	++	es	Some cystic foll. Several c.l.
1181	175	191	16	3	E	++	es	Small cystic c.l. Cystic foll.
1537	194	253	59	4	E	++	es	Many cystic c.l. Many foll.
2224	21	87	66	3	E	++	es	Few small c.l. Some foll.
2225	21	87	66	3	E	++	es	Few small c.l. Some foll.
2107	30	107	77	3	E	++	es	Few small c.l. Groups of foll.
2141	38	132	94	3	E	++	es	Small c.l. Small cystic foll.
2044	33	130	97	2	E	++	es	Small irregular c.l. Increased interstitial fib. tissue
2084	30	153	123	2	D	++	es	C.l. varying in size. Some cystic foll.
1170	32	179	147	6	E	++	es	Small c.l., some cystic. Cystic foll.
2714	25	176	151	5	E	++	es	Few distinct c.l. Numerous foll., some cystic.
1189	82	237	155	4	E	++	es	Multiple c.l., partially cystic. Few foll.
2739	28	229	201	4	E	++	es	Few c.l. Cystic foll.
2355	48	396	348	10	E	++	a	Two small c.l. No Foll. Biopsy at 164 days: c.l.
2552	47	500	453	6x5	E	++	es	Lobules of lut. cells. No distinct c.l. or foll.
2553	47	500	453	4	E	++	es	Several small c.l. Cystic foll. Increased interstitial fib. tissue

* The animals listed in this table had both ovaries removed, and either the entire or half of the left ovary inserted into the spleen. After the operation, adhesions developed between the spleen and the parietal abdominal wall, so that blood from the transplant entered the systemic circulation without passing through the liver. The vaginal smears of these animals contained cornified epithelial cells. The uterus showed estrogenic stimulation.

** D, diestrous; E, estrous

† +, adhesions to pancreas, omentum or mesentery; ++, adhesions to abdominal wall, kidney or uterine horn

†† a, atrophy; es, estrogenic stimulation

§ c.l., corpora lutea; fib., fibrous; foll., follicles; lut., luteal; str., structures; tr., transplant; tub., tubular

TABLE 2—*Continued*

TISSUE NO.	AGE AT		TRANSPLANT		PREDOMINANT TYPE OF VAGINAL SMEAR**	ADHESIONS†	UTERUS OR VAGINA††	PATHOLOGIC FIDINGS§
	Castration and Transplantation	Autopsy	Age	Size				
	days	*days*	*days*	*mm.*				
1526	24	627	603	4	E	++	es	Small cystic tr. with tub. formations. Biopsy at 103 days: small clusters of c.l.
1514	27	676	649	2	E	++	es	Minute tr. shows distorted clusters of lut. cells. No c.l. No foll.
1516	28	716	688	3	D	++	a	Minute cystic implant with tub. str., presumably lut.

liver without inactivation. In these animals, the constant estrogenic stimulation of the uterus sometimes produced severe cystic hyperplasia of the endometrial glands with focal metaplasia of the columnar epithelium to stratified squamous epithelium. The hypophysis often revealed a chromophobe hyperplasia of the anterior lobe (Fig. 14).

The animals that developed postoperative vascular adhesions between the transplant and systemic circulation served as one type of control for this experiment, and the data on these animals are listed in Table 2. These animals could be selected at an early period because the vaginal smear showed evidence of estrogenic stimulation that probably varied with the degree of vascularity of the adhesion. The pertinent data in Table 2 are listed under the same headings as in Table 1. An analysis of the histologic findings in these animals showed conclusively that typical luteomas or granulosa cell tumors were not formed. The transplants in these animals never achieved the size of the uncomplicated transplants, despite the fact that follicular growth apparently proceeded actively. The corpora lutea were formed from the follicles, but they commonly contained a central fibrous core, and usually were much smaller. As the transplant aged, the corpora lutea seemed to decrease in size and to lose their outlines, and there was even a tendency for them to coalesce (Fig. 15). This last feature bore some resemblance to the luteoma but it never revealed the masses of actively growing cells. Nests of granulosa cells were not found.

The observations after castration and transplantation of an ovary into the kidney are shown in Table 3. Histologic examination revealed ovarian tissue that was viable for periods of more than two years. These transplants maintained the uterus and vagina in an estrous state. Some of these transplants became cystic and they rarely exceeded a maximum diameter of 5 mm. During the first year these transplants were composed of about 20 small corpora lutea and occasional follicles. The cysts were derived from either the corpora lutea or the follicles, and generally were lined by fibrous tissue. The corpora were surrounded by a fibrous stroma and almost all contained a central fibrous core.

TABLE 3

TRANSPLANTATION OF AN OVARY TO THE KIDNEY OF CASTRATE FEMALE RATS*

TISSUE NO.	AGE AT		AGE OF TRANSPLANT	PREDOMINANT TYPE OF VAGINAL SMEAR**	UTERUS OR VAGINA†	PATHOLOGIC FINDINGS††
	Castration and Transplantation	Autopsy				
	days	*days*	*days*			
2368	24	29	5	E	es	Small fib. scar
2385	28	39	11	E	es	Developing foll. Small c.l.
2108	73	158	85	E	es	Large c.l. and developing foll.
2249	31	201	170	E	es	Many c.l. Few foll.
2307	33	312	279	E	es	Many small c.l. and small foll. cysts. No developing foll.
2551	31	404	373	E	es	Cystic c.l. Clusters lut. cells in wall of cyst. Few prim. foll.
2579	73	461	388	E	es	Poorly demarcated c.l. Scattered lut. cells in stroma. Distorted small foll.
2521	48	448	400	E	es	Poorly defined c.l. Clusters lut. cells in stroma
2808	38	464	426	E	es	Several cysts, poorly defined c.l. Tubular lut. str.
2528	33	453	420	E	es	Compactly arranged lut. cells in dense fib. tissue
2732	31	494	463	E	es	Cystic with clusters of lut. cells in wall. Few gran. cells
1549	68	569	501	E	es	Slightly cystic, tub. str. not distinct. Questionable foll. No. c.l.
1551	69	574	505	E	es	Cystic, two severely atrophic c.l. No foll. Tub. str. not distinct (adenocarcinoma of uterus)
2808	48	579	531	E	es	One distinct c.l. Scar tissue with lut. cells. Small cyst. Some pseudotub. str.
1176	73	620	547	E	es	Lut. cells irregularly distributed in dense fib. stroma. Tub. str.
2990	73	640	567	E	es	Multiloc. cysts, cords and tub. str. of small cells. No distinct lut. cells
1544	84	689	605	E	es	Cystic with tub. str. No. c.l. No foll.
1091	33	693	660	E	es	Multilocular cyst, cords and tub. str. of small cells. No distinct lut. cells. Single foll.
1543	25	708	683	E	es	Tub. str. No. c.l. No foll.
1175	33	717	684	E	es	Tub. str. No c.l. No prim. foll.
2057	48	768	720	E	es	Several c.l. and foll. No tub. str.
1542	28	767	739	E	es	Tub. str. No c.l. No foll.

* The animals listed in this table had both ovaries removed, and either the entire or half of the left ovary inserted into the kidney. The age of the animals at the time of operation, and the age of the animals at autopsy are indicated. The size of the transplant is not indicated because it varied from 2 to 6 mm. Accurate measurements were not readily obtained.

** E, estrous

† es, *estrogenic stimulation*

†† c.l., corpora lutea; fib., fibrous; foll., follicles; gran., granulosa; lut., luteal; prim., primordial; pseudotub., pseudotubular; str., structures; tub., tubular

Primordial follicles were extremely few in number. Examination of transplants that were between one and two years of age usually revealed slow disintegration of the corpora lutea with a separation of the cells by fibrous tissue. In the oldest transplants the corpora were usually indistinguishable. Cords and tubular formations of small cells appeared in the stroma (Fig. 16). The nature of these structures is not clear; they resemble the cords of epithelial cells that Hill[14] described in grafts of ovarian tissue to the ears of mice. There were no primordial follicles or ova. There was no evidence of the formation of a luteoma or granulosa cell tumor.

Hypophysectomy carried out at the time of castration and transplantation of the ovary into the spleen did not prevent the transplant from "taking". But there was no growth of the transplant and corpora lutea did not form. An example of the transplant in an animal prepared in this manner which survived for 222 days is shown in Figure 17. It was composed of a scant stroma in which were several atrophic follicles with granulosa cells, that had the typical "cart-wheel" arrangement of the chromatin. Injections of serum of a pregnant mare or the subcutaneous implantation of normal whole hypophyses stimulated the growth of the transplant, with formation of new follicles and corpora lutea.

DISCUSSION

Transplantation of an ovary into the portal circulation of a castrate rat alters the basic relationship between the ovary and the hypophysis. In the animals in which no adhesions formed between the implant and the systemic circulation, the ovarian hormones were inactivated by the liver, as indicated by a castrate type of vaginal mucous membrane. The hypophyses of these animals usually revealed castrate cells in the anterior lobes. These two observations were noted in our original report and it was suggested that at least two factors in the production of the tumors could be causative. These were continuous hypophyseal stimulation or removal of estrogenic inhibition of the ovarian transplant. That hypophyseal hormones were increased in amount was inferred from the presence of castrate cells in the hypophysis. Heller and Jungck[13] found that hypophyses removed from animals with intrasplenic ovarian transplants contained increased amounts of gonadotrophins, although not as much as the hypophyses of ordinary castrate rats. It seems permissible to infer that with the removal of the estrogenic hormones from the systemic circulation the hypophysis delivers a continuous and not a cyclic stimulation to the ovarian transplant. It is possible that the constant stimulation from the hypophysis, even if the amount of hormone is less than in the ordinary castrate, may have the effect of producing the unusual growth of the transplant. In addition, it may be that castration has altered the gonadotrophins qualitatively as well as quantitatively. If hypophyseal stimulation is the more important factor, then this is the first demonstration that prolonged intensified endogenous hypophyseal stimulation of an ovary may produce changes that have all the characteristics of neoplasm. Related evidence is a report by Evans, Simpson and Li[9] who found 3 small ovarian tumors in a series of rats that had been injected regularly for 437 days with purified growth hormone derived

from the hypophysis. The tumors were very small, the cell type resembled the cells of a luteoma, but the pattern suggested an arrhenoblastoma.

The inactivation of the endogenous ovarian hormones by the liver presents another possible etiologic factor. The estrogens elaborated by the ovary may reach the components of the ovary by permeation through the tissues from its site of origin, or the estrogens may be secreted directly into the blood stream and return to the ovary by the same route. Estrogens are considered to be produced by the follicle cells, the theca interna and the corpus luteum. It has been suggested that estrogen secreted by the theca interna may permeate through the follicle and aid in its growth.[15] On the other hand, these experiments suggest that the estrogens leave the ovarian transplant by way of the circulation and are destroyed before they can return. Thus the ovary is no longer influenced by its endogenous hormones, and one factor that may have had some control over the growth of the follicles and corpora lutea is absent. The administration of exogenous estrogen in the form of a pellet or by injection[13] inhibits the growth of the transplant; however, it is not possible to separate the effect of the estrogen on the hypophysis from its effect on the transplant. From the material examined it was not possible to state which of the two mechanisms, continuous hypophyseal stimulation or removal from estrogenic inhibition, was the more important; in fact, other unknown conditions might be the primary factors.

In addition to demonstrating that alterations in the normal hormonal balance may lead to neoplastic changes, these experiments substantiate current concepts of ovogenesis.[15] Serial sections of the transplanted ovaries made 24, 48, and 72 hours after transplantation showed no recognizable surviving tissue. The primordial follicles, graafian follicles, and, in the case of the adult ovary, the corpora lutea, undergo infarction and necrosis, and subsequently the necrotic remnants are removed by the inflammatory elements. Although surviving ovarian tissue is not recognizable as such, the follicles that develop after the inflammatory reaction subsides are considered to arise from remnants of the germinal epithelium or individual stromal cells that must have survived the transplantation. The earliest follicles are scattered at the junction of the transplanted tissue and spleen, a site where germinal epithelium would be likely to survive. The presence of primordial follicles developing among the corpora lutea in the inner portions of the older transplants suggests that some germinal tissue had been forced into the transplant by the growth of the first follicles. In the transplant the germinal epithelium gives rise to the primordial follicles with regularity over a period of at least five months. The exact number of follicles thus formed has not been estimated, but there seems to be no apparent difference in the number that arise from transplants of one-half ovary compared to transplants of a whole ovary, whether immature or mature at the time of transplantation. There is no explanation why all the surviving germinal epithelium does not form follicles immediately. The factors that permit certain few follicles to develop and luteinize at one time are not evident. The sections of transplants up to five months of age show follicles in all stages of development. An interesting observation is that follicles fail to develop in the transplant that has undergone the transforma-

tion into the luteoma. It is not known if there is some specific effect that either prevents further development of new follicles, or stimulates the luteoma which then may inhibit the growth of the follicles. It may be that the luteoma cannot form until all the follicles have developed.

The failure of involution of the corpora lutea during the first phase of the growth of the transplant is probably the result of the hormonal imbalance. In the control groups, particularly the one with the ovarian transplant in the spleen plus adhesions, or with the transplant in the kidney, involution of the corpora lutea takes place but to a variable degree and only after long periods. Luteoma does not occur in these groups. It is possible that the same factors that maintain the corpora lutea in our experimental animals may be related to those that maintain the corpora lutea during pregnancy or in the presence of a tumor that produces chorionic gonadotrophins. Since in our experimental animals there is no site for the production of chorionic gonadotrophins, stimulation from the castrate hypophysis may be implicated. In the animals that have one intact normal ovary and an ovarian transplant in the spleen, the hypophyseal hormones are sufficient to maintain normal ovarian function in the intact ovary, but do not stimulate the ovary in the spleen.[1] If these animals become pregnant, the ovary in the spleen reveals slight growth and many active follicles appear despite the presence of estrogen from the normal ovary and from the pregnancy.[2] Thus it is suggested that the constant hypophyseal stimulation is probably the most important factor in the maintenance of the corpora lutea.

The cellular growth that we have designated luteoma appears at a relatively specific interval after transplantation and has the histologic characteristics of a neoplasm. The structure of a congeries of corpora lutea has been superseded by a mass of actively growing cells that still maintain the appearance of individual luteal cells. The origin of the luteoma from one or more corpora lutea is suggested by the presence of a small growing nodule of these cells as one component in a transplant otherwise composed of distinct corpora lutea. This nodule is often surrounded by, and finally pushes aside, the corpora lutea that have not undergone this peculiar transformation. Some further hormonal alteration takes place at the time the luteoma appears because, no longer is it possible to find follicles in any stage of development. Also, in some animals, the luteoma produces sufficient estrogen to pass through the liver and to stimulate the vagina and uterus; it may be that even in the animals in which this estrogenic activity is not grossly evident, estrogen from the luteoma permeates the transplant and inhibits the further development of follicles.

The factors responsible for the formation of the granulosa cell tumors are as obscure as the origin of the granulosa cells in the luteoma. It is possible that an occasional primordial follicle may have been trapped in the growing luteoma and after a long dormant interval proliferates into large nests of actively growing cells. It is difficult to consider luteal cells the precursors of the granulosa cells unless some form of dedifferentiation takes place. However, the multicentric distribution of the granulosa cell clusters in the luteomas, and the presence of nests and masses of cells that were neither typically luteal nor definitely granu-

losa suggest that some unusual factors influenced the formation of the granulosa cell tumors. It is interesting to note that in many of the granulosa cell tumors either so much estrogen is produced that the liver can no longer inactivate it or a form of estrogen that cannot be inactivated by the liver is formed. The estrogen enters the systemic circulation and cornifies the cervix and vagina, changes the hypophysis but seems to have no effect on the tumor itself. This latter fact stands out sharply when compared to the inhibiting effect of estrogen on the growing transplant.

SUMMARY AND CONCLUSIONS

An experimental ovarian tumor is produced in castrate rats by transplanting either one-half or the whole ovary into the spleen. A definite sequence of changes takes place after this transplantation. This is initiated with partial infarction of the transplanted ovary and with concomitant inflammatory reaction and is followed by healing. Some of the transplanted ovarian tissue survives and from this there is a continuous formation of primordial follicles that develop and undergo luteinization. The corpora lutea do not regress or involute; thus the mass of transplanted tissue steadily increases in size. As early as 157 days, there appear neoplastic luteal cells in the transplant, and ultimately these compress and replace the corpora lutea. Luteoma appeared in each of the 36 transplants that were permitted to live 317 days or longer. A granulosa cell tumor developed in 24 of the 46 luteomas and in some instances entirely replaced the luteoma. The granulosa cell tumor had most of the histologic characteristics commonly seen in the types encountered in human beings. The luteomas and the granulosa cell tumors occasionally produced sufficient estrogen to pass through the liver and to enlarge the uterus and cornify the vagina. Neither the luteomas nor the granulosa cell tumors metastasized.

A major factor concerned in the etiology of this experimental tumor in rats is the inactivation by the liver of the hormones produced by the transplanted ovary. This may result either in a release of the transplanted ovary from its own inhibiting hormones, or in a constant stimulation of the transplanted ovary by the hypophysis, or in both factors working simultaneously. This experiment illustrates that in the rat a tumor with the histologic characteristics of a neoplasm can result from an imbalance of endogenous hormones.

Acknowledgment. The authors are indebted to Richard Pencharz, Ph.D., for his technical collaboration.

REFERENCES

1. Biskind, G. R., and Biskind, M. S.: Atrophy of ovaries transplanted to the spleen in unilaterally castrated rats; proliferative changes following subsequent removal of intact ovary. Science, **108:** 137–138, 1948.
2. Biskind, G. R., and Kordan, B.: In preparation for publication.
3. Biskind, G. R., and Mark, J.: Inactivation of testosterone propionate and estrone in rats. Bull. Johns Hopkins Hosp., **65:** 212–217, 1939.
4. Biskind, G. R., Pencharz, R., and Biskind, M. S.: The pathogenesis of intrasplenic ovarian tumors in rats. Proc. 4th Internat. Cancer Res. Congress, 1947, in press.
5. Biskind, M. S., and Biskind, G. R.: Effect of vitamin B complex deficiency on inactivation of estrone in the liver. Endocrinology, **31:** 109–114, 1942.

6. BISKIND, M. S., AND BISKIND, G. R.: Inactivation of testosterone propionate in the liver during vitamin B complex deficiency; alteration of the estrogen-androgen equilibrium. Endocrinology, **32**: 97–102, 1943.
7. BISKIND, M. S., AND BISKIND, G. R.: Development of tumors in the rat ovary after transplantation into the spleen. Proc. Soc. Exper. Biol. and Med., **55**: 176–179, 1944.
8. BISKIND, M. S., AND SHELESNYAK, M. C.: Effect of vitamin B complex deficiency on inactivation of ovarian estrogen in the liver. Endocrinology, **30**: 819–820, 1942.
9. EVANS, H. M., SIMPSON, M. E., AND LI, CHOH HAO: The gigantism produced in normal rats by injection of the pituitary growth hormone; body growth and organ changes. Growth, **12**: 15–32, 1948.
10. FURTH, J.: Transplantability of induced granulosa cell tumors and of luteoma in mice; secondary effects of these growths. Proc. Soc. Exper. Biol. and Med., **61**: 212–214, 1946.
11. FURTH, J., AND SOBEL, H.: Neoplastic transformation of granulosa cells in grafts of normal ovaries into spleens of gonadectomized mice. J. Nat. Cancer Inst., **8**: 7–16, 1947.
12. GOLDEN, J. B., AND SEVRINGHAUS, E. L.: Inactivation of estrogenic hormone of the ovary by the liver. Proc. Soc. Exper. Biol. and Med., **39**: 361–362, 1938.
13. HELLER, C. G., AND JUNGCK, E. C.: Regulation of ovarian growth; inhibition by estrogen or stimulation by gonadotrophins. Proc. Soc. Exper. Biol. and Med., **65**: 152–154, 1947.
14. HILL, R. T.: Fate of ovaries which have been grafted in the ear for long periods of time. Endocrinology, **28**: 426–430, 1941.
15. HISAW, F. L.: Development of the graafian follicle and ovulation. Physiol. Rev., **27**: 95–119, 1947.
16. JUNGCK, E. C., HELLER, C. G., AND NELSON, WARREN O.: Regulation of pituitary gonadotrophic secretion; inhibition by estrogen or inactivation by the ovaries. Proc. Soc. Exper. Biol. and Med., **65**: 148–152, 1947.
17. LI, M. H.: Malignant granulosa cell tumor in an intrasplenic ovarian graft in a castrated male mouse. Am. J. Obst. and Gynec., **55**: 316–320, 1948.
18. LI, M. H., AND GARDNER, W. U.: Tumors in intrasplenic ovarian transplants in castrated mice. Science, **105**: 13–15, 1947.
19. LI, M. H., AND GARDNER, W. U.: Granulosa cell tumors in intrapancreatic ovarian grafts in castrated mice. Science, **106**: 608–609, 1947.
20. LIPSCHÜTZ, A.: Study of the gonadotrophic activity of the hypophysis in situ. Nature, London, **157**: 551, 1946.
21. LIPSCHÜTZ, A., PONCE DE LEON, H., WOYWOOD, E., AND GAY, O.: Intrasplenic ovarian grafts in guinea pig and problem of neoplastic reactions of graft. Rev. canad. de biol., **5**: 181–198, 1946.
22. PECKHAM, B. M., GREENE, R. R., AND JEFFRIES, M. E.: Granulosa cell tumors in female rats and rabbits. Science, **107**: 319–320, 1948.

ON THE NEUROGENESIS OF SO-CALLED GRANULAR CELL MYOBLASTOMA*

JOHN A. FUST, M.D., AND R. PHILIP CUSTER, M.D.†

From the Laboratories of the Presbyterian Hospital in Philadelphia, Pennsylvania, and The Army Institute of Pathology, Washington, D. C.

Most of the discussion of so-called granular cell myoblastoma in the medical literature has been concerned with their nature, controversy having revolved especially around three questions: (1) Are the lesions true tumors or are they the result of a myolytic process? (2) Are the granular cells derived from pre-existing voluntary muscle or do they arise *de novo*? (3) Why are these tumors observed so frequently in regions normally devoid of voluntary muscle? That they are truly myogenous has been accepted by most authors.

The extensive review of Crane and Tremblay,[3] and the recent report of Simon[10] make a full survey of the literature unnecessary, but there are a few points worthy of reiteration. The tumors were first described by Abrikossoff (1926),[1] and termed "Myoblastenmyome", since he thought that their cells resemble the primitive skeletal muscle cells drawn by Godlewski (1902).[5] All 5 tumors in his original series arose in connection with voluntary muscle; 3 were in the tongue, and one each in the lip and gastrocnemius muscle. It was Abrikossoff's first impression that the tumor cells are primitive myoblasts developing in response to injury. Klinge's[8] series included 2 tumors in the skin, which he explained on the basis of muscle rests; his hypothesis was later approved, but not proved, by Abrikossoff (1931)[2] and other writers.

Several observers, including Abrikossoff, described apparent transition between mature muscle fibers and tumor cells. This has been disputed, however, most emphatically by Klemperer,[7] who stated, ". . . Schirmer and Meyer correctly maintained that such findings were most likely due to erroneous interpretations of the relation between neoplastic elements and pre-existing striated muscle fibers." Roffo,[9] Gray and Gruenfeld,[6] and others suggested that the lesions, especially those of the tongue, represent simply a degenerative process in muscle. Gray and Gruenfeld[6] regarded as unwarranted the comparison between these tumors and embryonal skeletal muscle and proposed that the term myoblastoma be dropped. They were satisfied, however, that the lingual tumors are myogenous, but were not convinced that this is true of the extralingual ones.

We[4] went one step further (1948) by demonstrating the neurogenesis of 34 "granular cell myoblastomas" located in the skin and subcutaneous tissue, and one each in the larynx and tongue. At that time we regarded 15 other lingual

* Published under the auspices of The Surgeon General, U. S. Army, who does not necessarily assume responsibility for the professional opinions expressed by the authors.

Received for publication, March 4, 1949.

† Dr. Fust is Resident Physician in Pathology, Presbyterian Hospital, and Dr. Custer is Director of Laboratories of the Presbyterian Hospital and Assistant Professor of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

tumors as myogenous. Restudy of the material has disclosed evidence in support of our present view, that *there is no essential difference between the lingual and extralingual tumors, and that they are all of neural origin.*

MATERIAL

The present series comprises 52 tumors originally diagnosed "granular cell myoblastoma" at either The Army Institute of Pathology or the Presbyterian Hospital. Thirty-five arose in the skin or subcutaneous tissue, one in the larynx, and 16 in the tongue (Table 1). In most cases sections were stained with hematoxylin and eosin, and impregnated by the Bodian technic, while fat and various connective tissue stains were available in many instances. The Bodian preparations were completely satisfactory only with freshly fixed and blocked tissue.

We did not include 4 cases of so-called congenital epulis, even though their histologic appearances are somewhat similar to the tumors under consideration.

TABLE 1

SITES OF TUMORS

Lip	3
Tongue	16
Vocal cord	1
Face	1
Neck	6
Chest wall	10
Abdominal wall	6
Perianal region	3
Buttock	1
Extremities, upper	3
Extremities, lower	2
Total	52

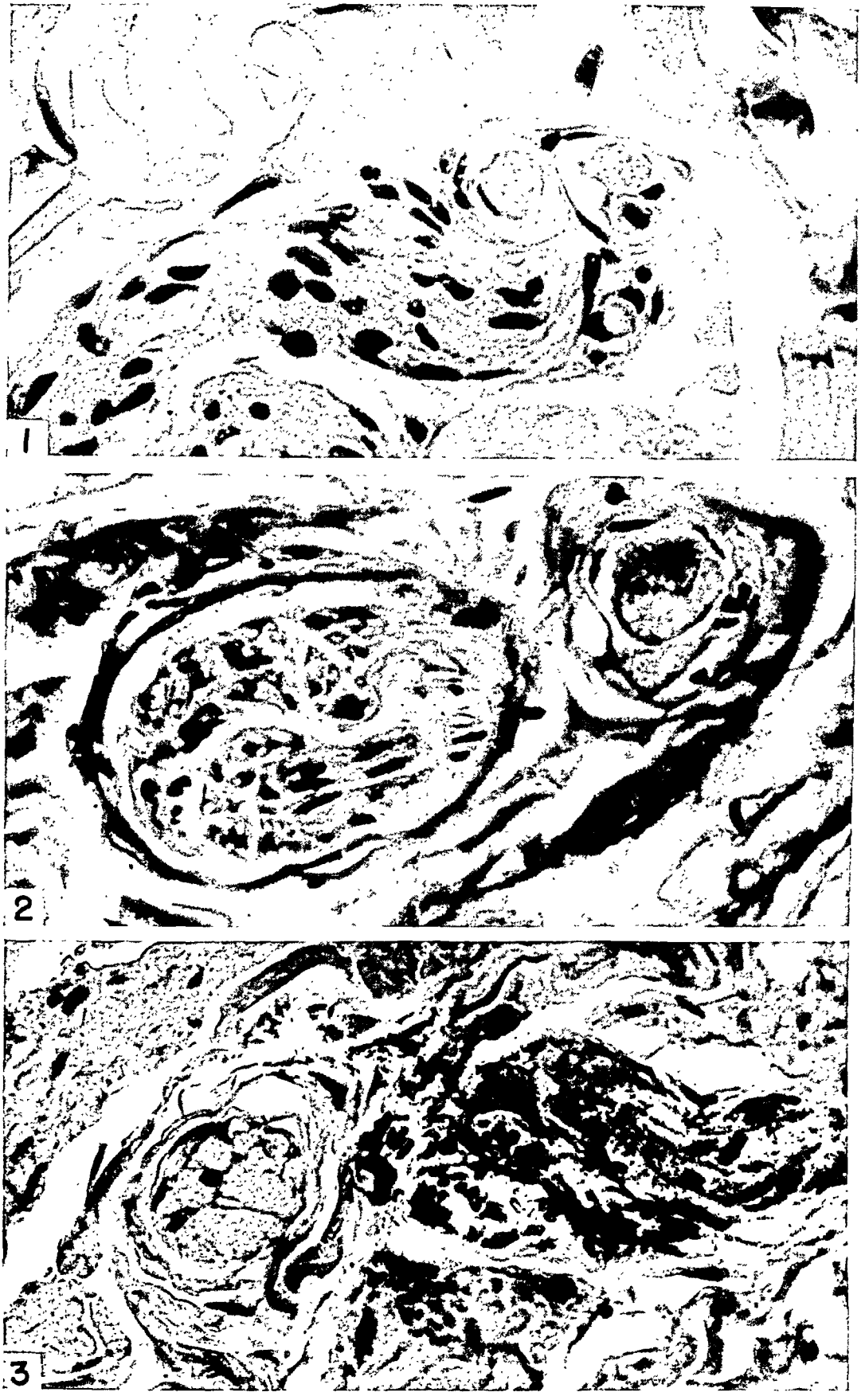
We were unable to demonstrate nerve components in them, and suspected that they might prove to be a separate neoplastic entity.

OBSERVATIONS

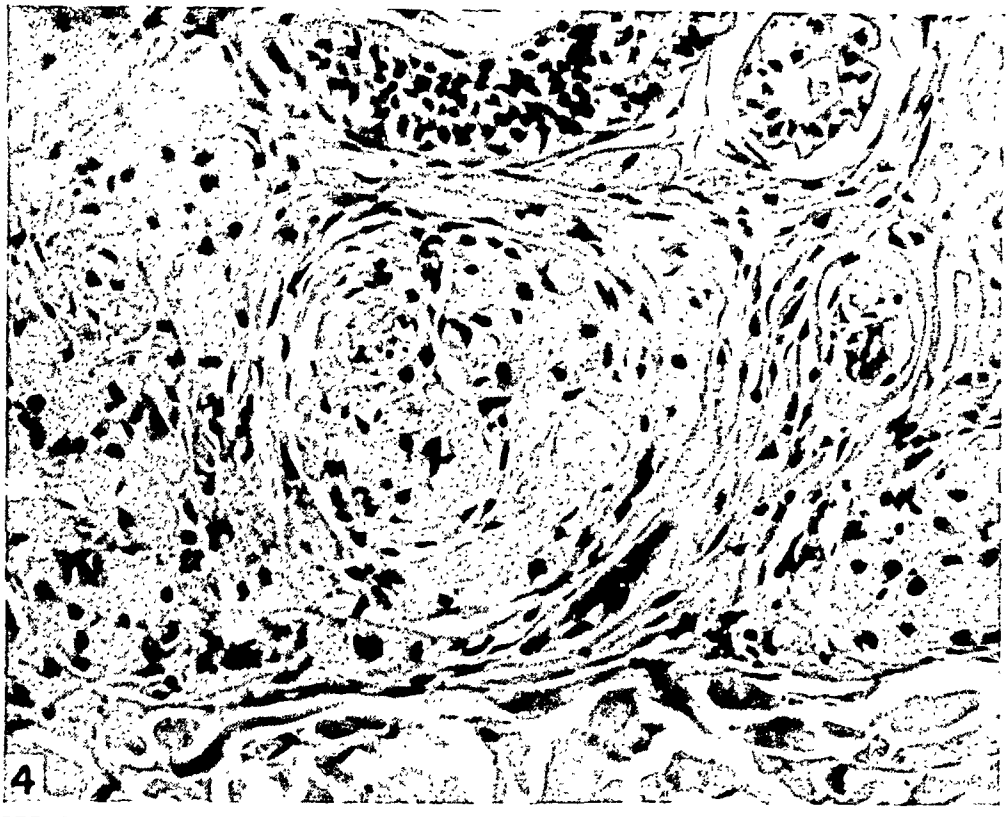
Because previous discussion has centered around lingual versus extralingual tumors, and those arising apart from striated muscle, we have divided our series accordingly.

Extralingual granular cell tumors. Study of the group bearing *no anatomic relation to skeletal muscle* first gave us the impression that these neoplasms arise from nerves, not muscle. It was noted that nerve twigs lying well beyond the limits of the masses contained large cells with granular cytoplasm, indistinguishable from cells of the tumors proper (Figs. 1–3). These distant cells were encompassed by the nerve sheaths, and appeared to be autochthonous rather than to represent tumor infiltration along the nerves.

At the margins of many of the tumors, coarse finger-like extensions ramified



FIGS. 1–3. Nerve twigs containing large cells with granular cytoplasm lying well beyond the reaches of three of the skin tumors. The cells resemble those of the tumors proper. All × 500. A.I.P. Negs. 103575, 103583, 103588.



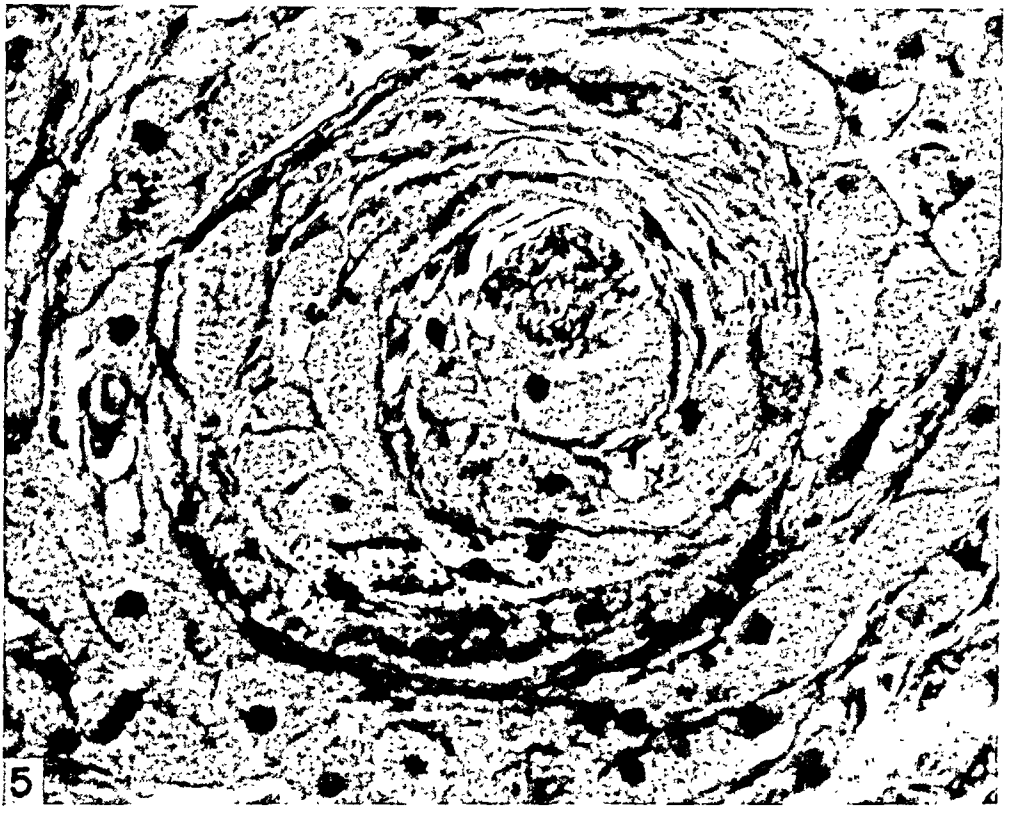


FIG. 4. Whorl of granular tumor cells centering about a core of axis cylinders and bordered by frayed collagen, apparently the residuum of nerve sheath. Such structures were found within both lingual and extralingual tumors. × 250. A.I.P. Neg. 103572.

FIG. 5. Same type of neural remnant at higher magnification and impregnated by the Bodian technic. × 600. A.I.P. Neg. 103574.

through contiguous tissue for varying distances (Figs. 6 and 7), nearly always centering about a bundle of axis cylinders and having a border resembling nerve sheath. Such structures continued into the solid portion of the tumors, still recognizable by reason of the nerve core and expanded sheath (Figs. 4 and 5). Other tumors with identical cytologic characters did not have these clearly defined nerve connections, and were composed of loose strands of large granular cells lying at random. Still others had very sharply defined borders and compact structure (Figs. 8–10), and most of these had demonstrable nerve components.

Four tumors in this group served to illustrate gradations from the conventional neurofibromatous pattern with relatively few granular cells to one in which granular cells predominated, although the fascicular structure persisted. The two extremes are illustrated in Figures 13 and 14.

Extralingual tumors *adjacent to striated muscle* were sometimes sharply demarcated (Fig. 10), but occasionally extended into the muscle (Fig. 11). In the latter case the tumor cells appeared to be guided into lines parallel with the muscle fibers to give the false impression of having arisen from them. Degenerative changes occurred in the involved muscle, although not to the extent that the fibers mimicked granular tumor cells as they did in the tongue (Figs. 15–18). These tumors were identical histologically with ones quite unrelated to muscle.

Specifically, neural elements were clearly demonstrable in 28 and questionable in 4 of 36 extralingual tumors.

Lingual granular cell tumors. Only one tumor of the tongue did not involve muscle, and it was polypoid and had a narrow pedicle (Fig. 8). Its histologic structure was that of the others in the group except that nerve trunks did not appear in the levels sectioned.

The 15 remaining tumors were embedded in striated muscle and had produced in it the degenerative changes that have been so misleading to us and others in the past. The myofibrillae had become fragmented to form fine granules, with the result that the sarcoplasm strikingly resembled the cytoplasm of the tumor cells (Figs. 15 and 16). This change was often patchy, so that, in longitudinal and cross section, both granules and well preserved myofibrillae were seen. Such degeneration occurred at the tumor margins and in residual muscle fibers encompassed by the tumor. In the latter location true tumor cells overlapped muscle fibers cut longitudinally or slightly tangentially, thus creating the impression of a histogenetic relationship (Fig. 16). To add to the deception, the contour of muscle fasciculi was frequently preserved, although muscle fibers were replaced by invading tumor cells (Figs. 17, 18 and 20).

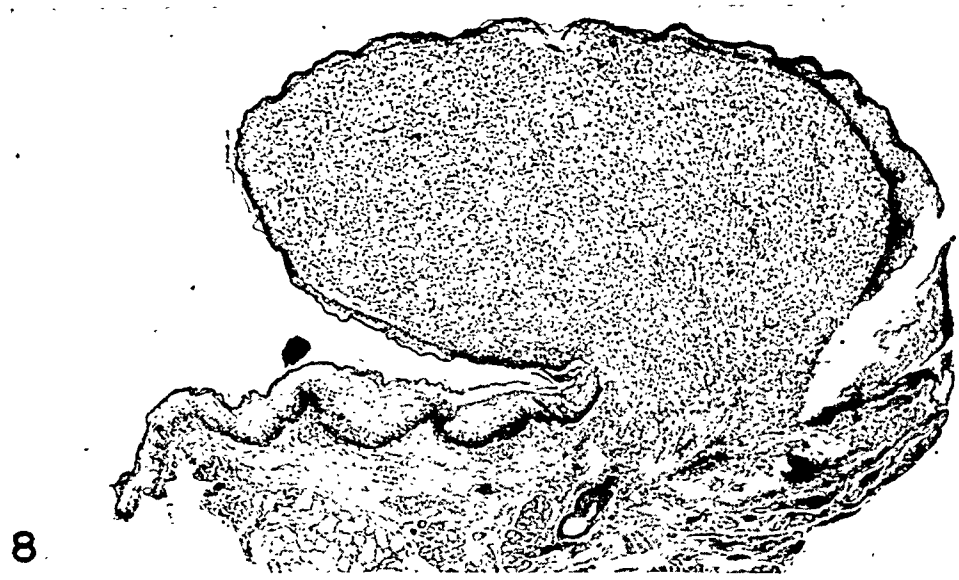
In sections stained with hematoxylin and eosin the granules of degenerated myofibrillae were almost identical with those of the neoplastic cells. In material prepared by the Bodian technic, however, they were decidedly unlike, the granules of the tumor cells being far less heavily impregnated than those of the myofibrillae. By this technic we also demonstrated what we at first believed to be very large granular tumor cells with a single compact nucleus and distinct intracytoplasmic septa (Fig. 12 A). Sections through the long axes of similar structures, however, showed that they were fasciculi of tapering interdigitated





FIG. 6. A coarse finger of tumor extending beyond the main mass. Neural elements (N) course through the center. × 100. A.I.P. Neg. 103579.

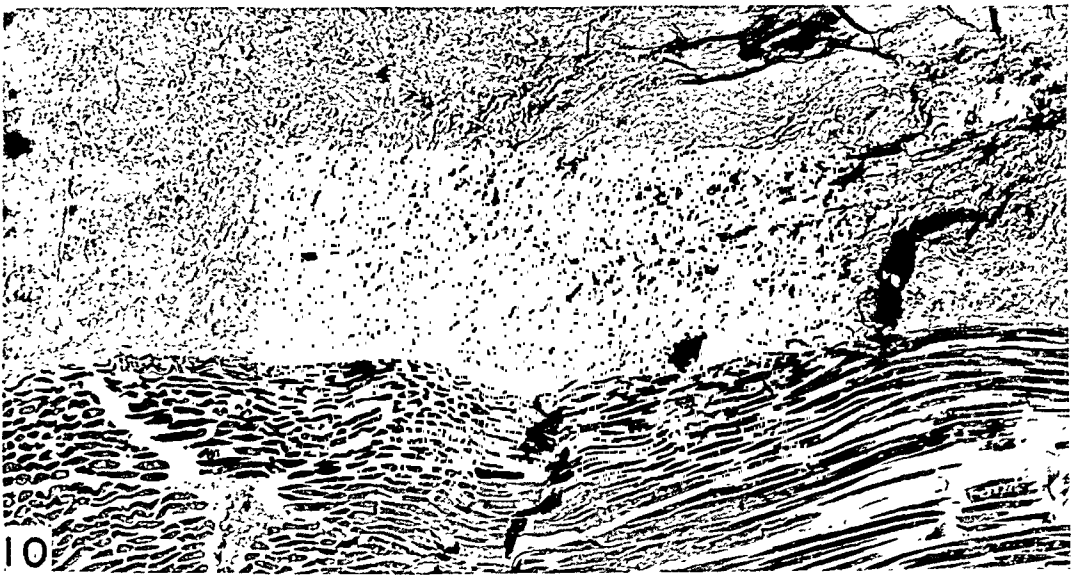
FIG. 7. The intimacy of tumor (T) and nerves (N) is clearly evident in the deep reaches of a "myoblastoma" of the skin. × 100. A.I.P. Neg. 103578.



8



9

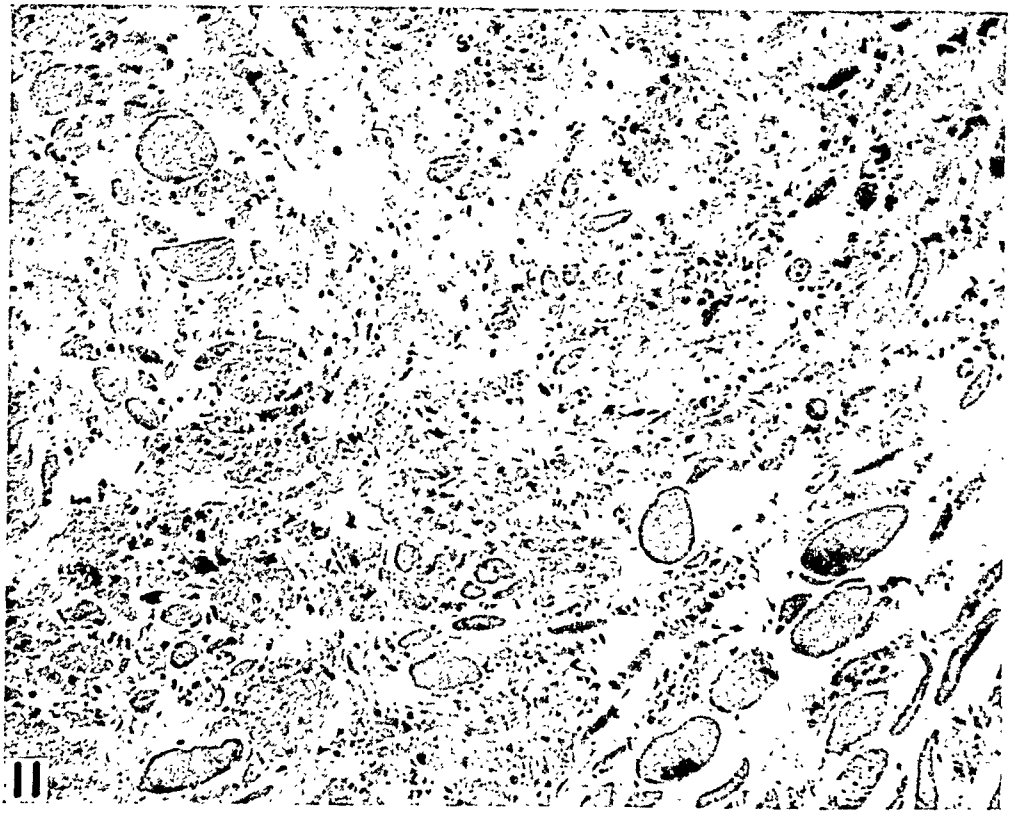


10

FIG. 8. A polypoid granular cell tumor of the tongue. This was the only lingual lesion that did not involve or even touch striated muscle. × 20. A.I.P. Neg. 103570.

FIG. 9. This lenticular mass arose in the skin of the buttock quite apart from voluntary muscle. One finds this configuration so frequently among ordinary neurofibromas of the skin. × 20. A.I.P. Neg. 103577.

FIG. 10. A granular cell tumor of pectoral sheath is, for the most part, sharply demarcated from the muscle. × 20. A.I.P. Neg. 103569.



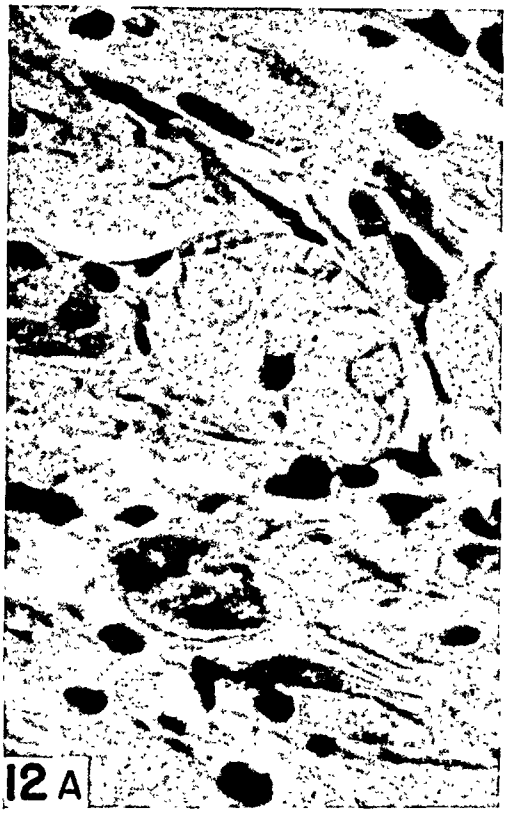
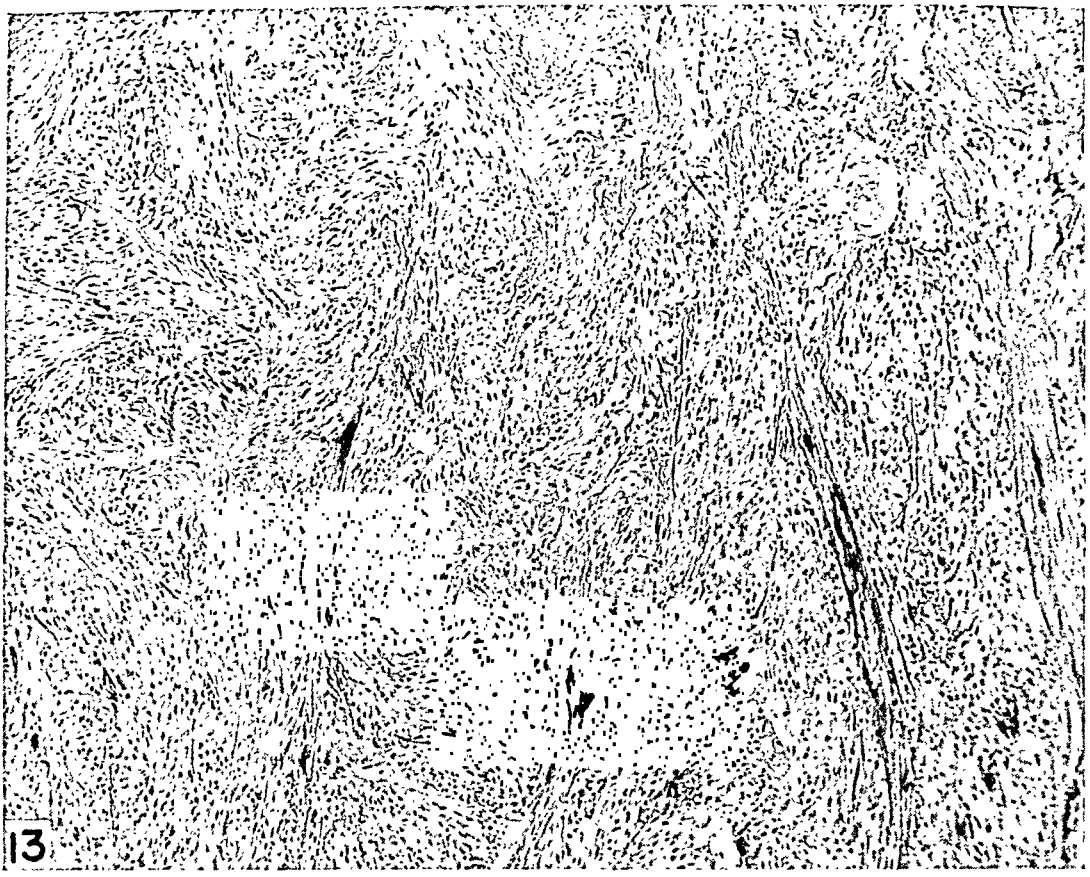




FIG. 11. From same tumor as Figure 10, showing extension into muscle, but without pseudotransitions found in lingual lesions. × 250. P. H. Neg.

FIG. 12. A. Structure in center appears as a large granular tumor cell with cytoplasmic "septa." × 900. (Bodian technic.) P. H. Neg.

B. Section through long axis of such a structure shows the "septa" to be cell membranes of interdigitated fusiform cells. Note also how granules in disintegrating muscle fibers (M) are more distinctly impregnated than those of tumor cells. × 900. (Bodian technic.) P. H. Neg.



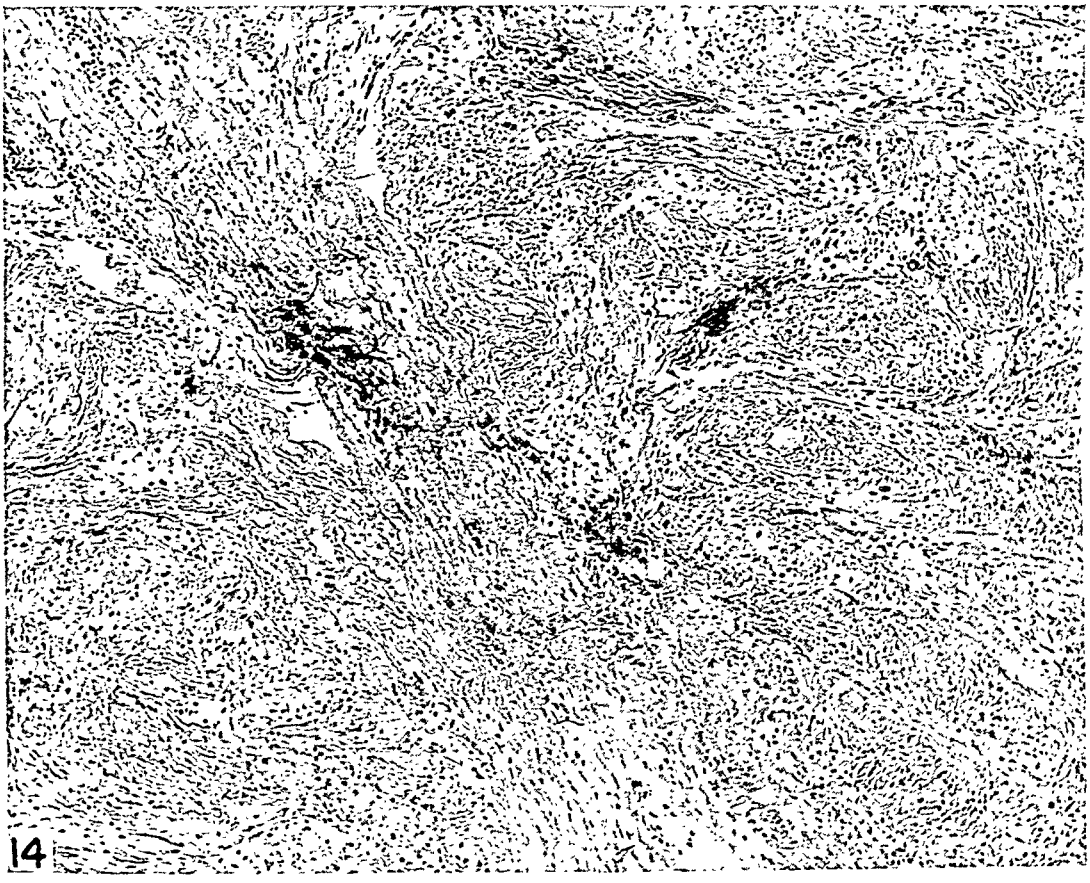


FIG. 13. The conventional pattern of a neurofibroma is seen in this tumor, although granular tumor cells were sparsely interspersed among the fasciculi. × 75. A.I.P. Neg. 103568.

FIG. 14. A similar tumor except that granular tumor cells were richly distributed and are visible even at this low magnification. × 75. A.I.P. Neg. 103571.



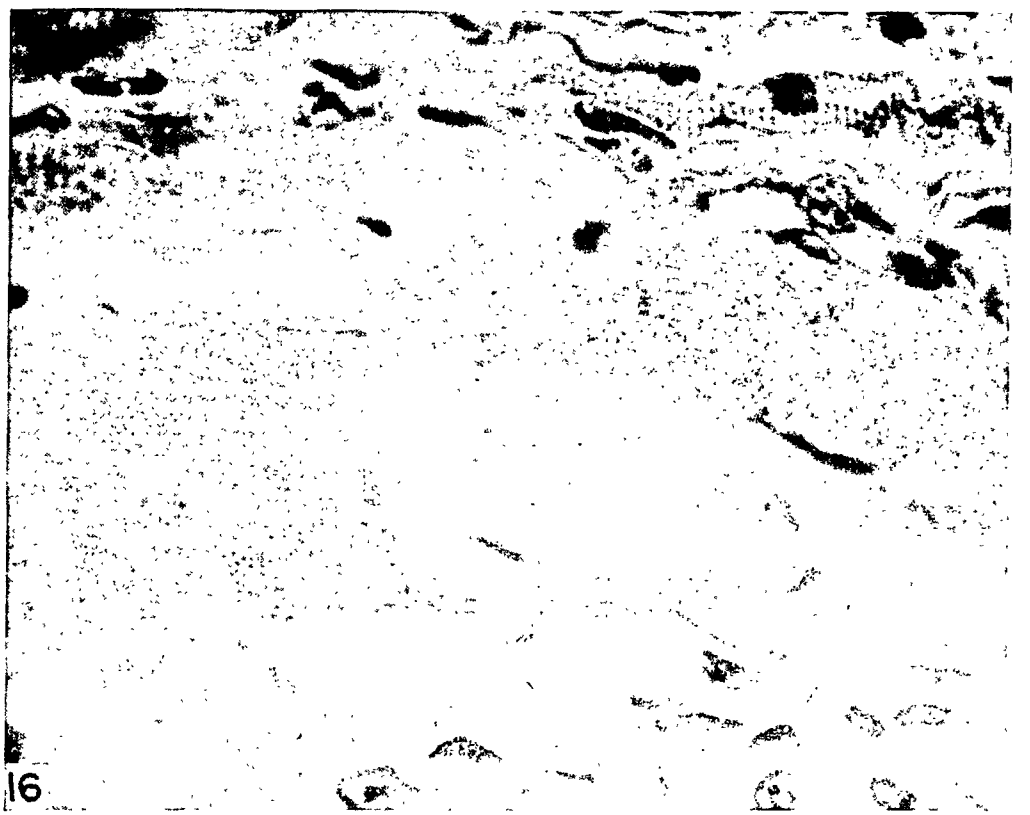
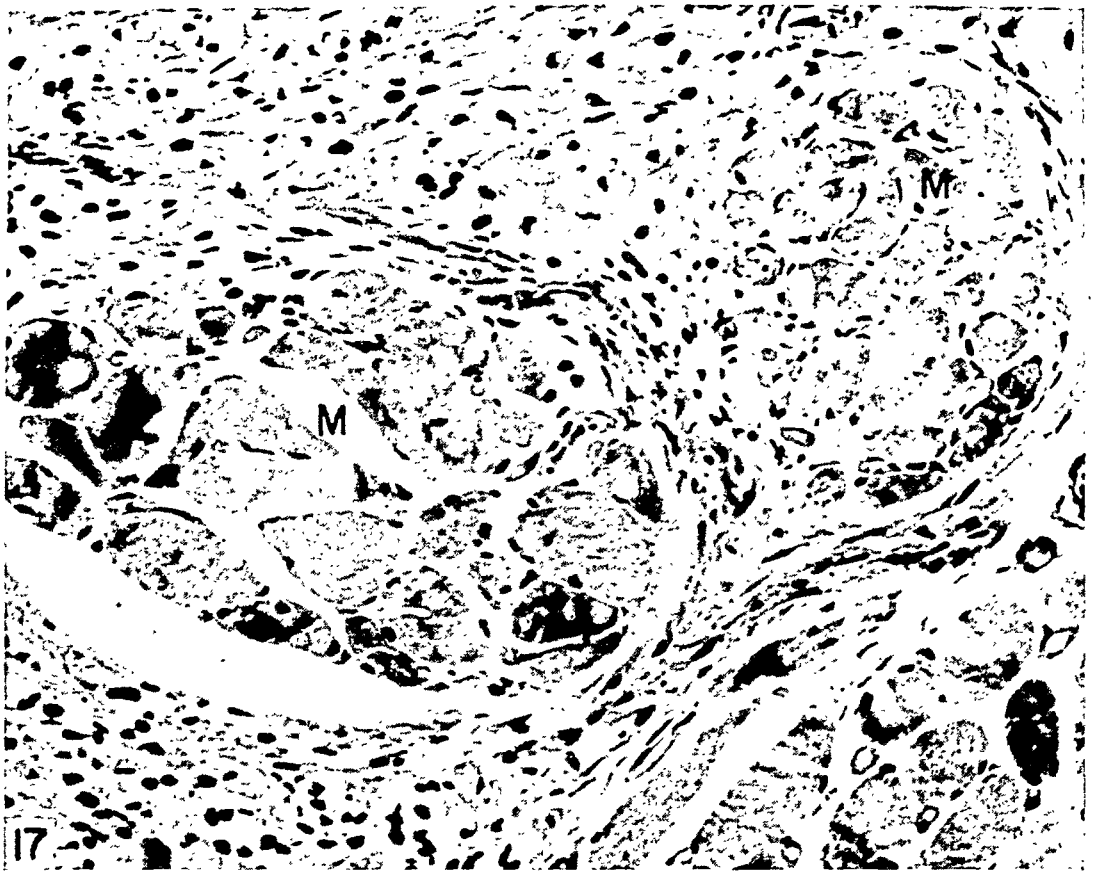


FIG. 15. Degenerative changes in muscle adjacent to a lingual tumor, ranging from little disturbed myofibrils to diffuse granularity. × 900. (Bodian technic.) A.I.P. Neg. 100316.

FIG. 16. Pseudotransitions between degenerated muscle fibers of the tongue and granular tumor cells, due to slightly tangential plane of section. × 900. (Bodian technic.) A.I.P. Neg. 100318.



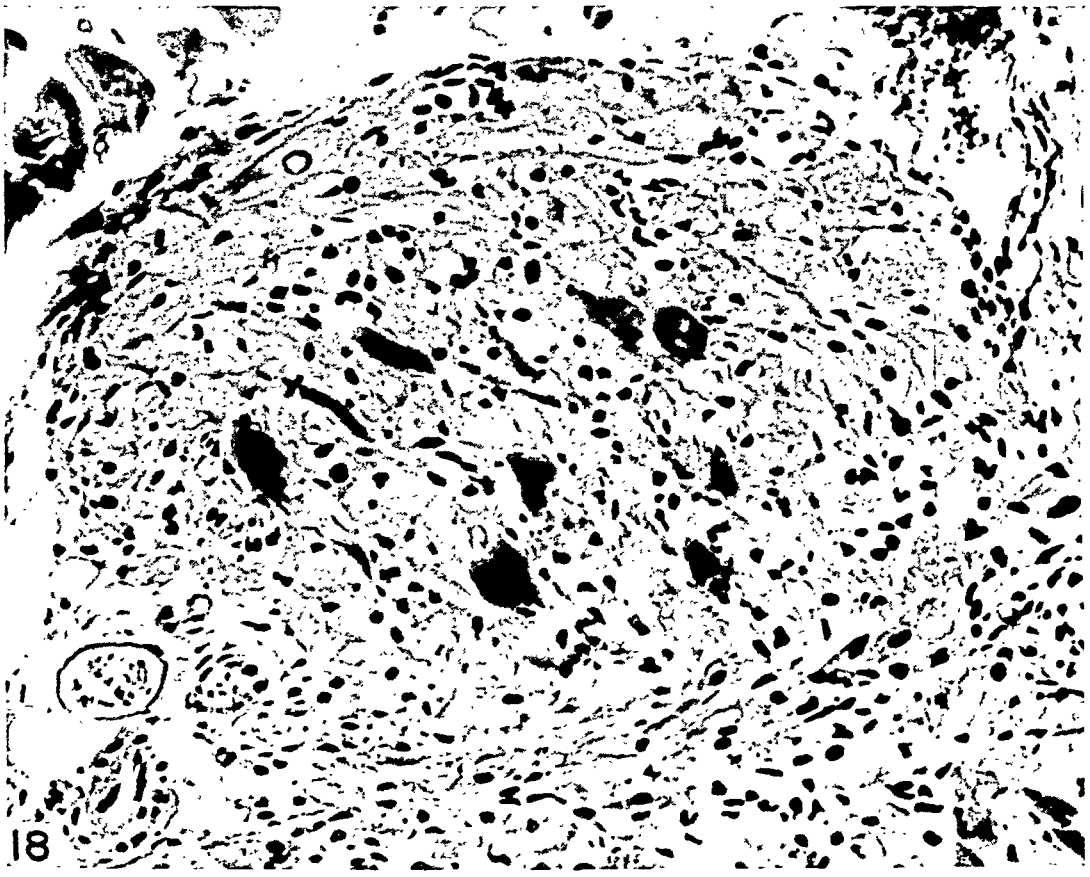
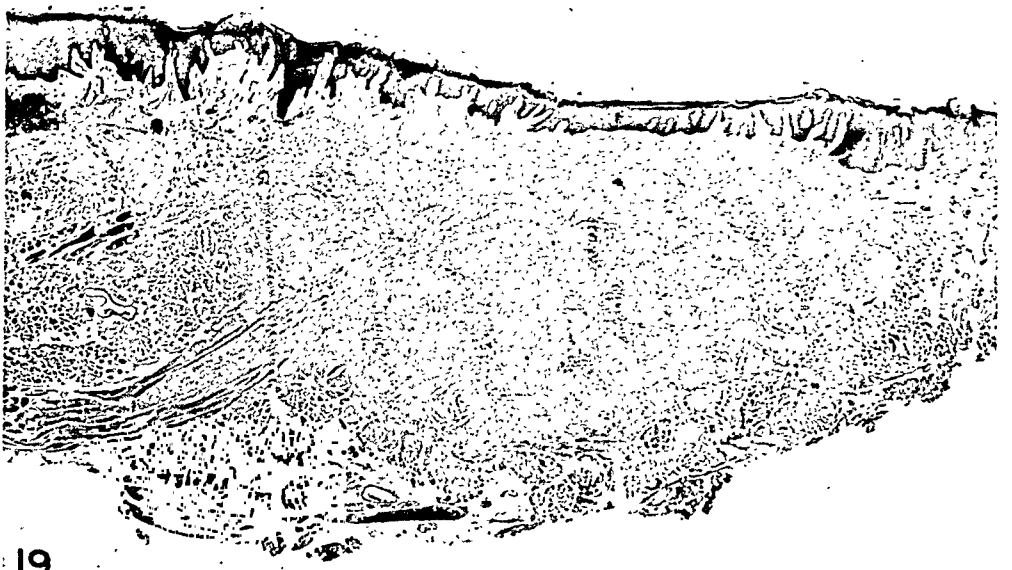
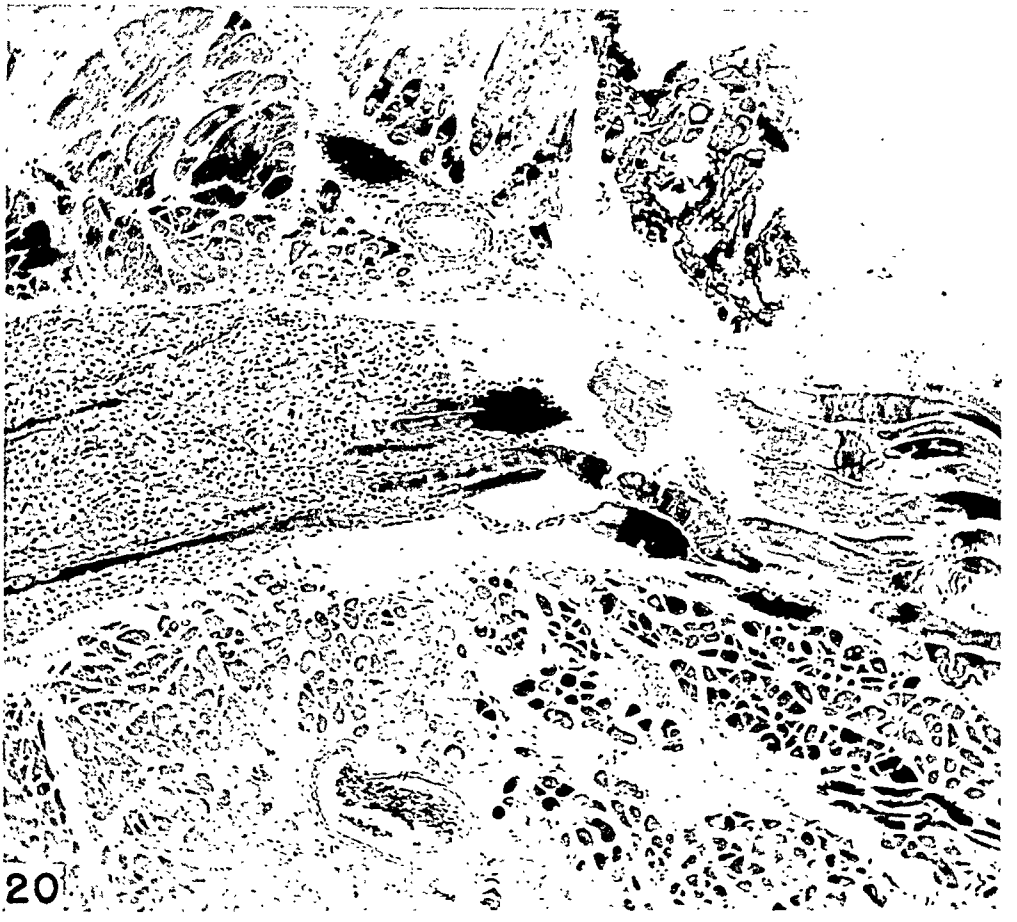


FIG. 17. Granular degeneration in muscle fibers (M) of the tongue cut in cross section and at an angle. Impinging granular cell tumor seen in upper and lower left. × 250. (Bodian technic.) A.I.P. Neg. 100329.

FIG. 18. Lingual tumor in which the cells have virtually replaced involved muscle save for a few residual fibers. The contour of the original muscle fasciculus is preserved. × 250. (Bodian technic.) A.I.P. Neg. 100311.



19



20

FIG. 19. Granular cell "myoblastoma" of the tongue. A false impression of myogenesis is gained by the manner in which the fasciculus in the lower left melts into the tumor. × 20. A.I.P. Neg. 103567.

FIG. 20. Higher magnification of the muscle fasciculus mentioned above, showing partial replacement by tumor and granular degeneration of nearby muscle. × 75. A.I.P. Neg. 100313.

tumor cells (Fig. 12 B), the nucleus of one having fortuitously appeared in the level of cross-section.

Five of the 15 tumors lying in lingual muscle contained the obviously neural structures that were so prominent in the extralingual group. These consisted of bundles of axis cylinders surrounded by concentric whorls of granular cells, the latter interleaved with fibrous strands resembling frayed nerve sheaths, exactly as shown in Figures 4 and 5. Such structures could not be demonstrated at the levels sectioned in the other 10 tumors, although they were similar in all other respects.

Malignant Quality

Not a single granular cell tumor in our series showed any evidence of malignant change. Reactions in the overlying stratified squamous epithelium varied from atrophy to pseudoepitheliomatous hyperplasia, and in 6 cases to actual squamous cell carcinoma. There was no significant difference between the lingual and extralingual group in this respect, one carcinoma having occurred in the tongue, one in the vocal cord, and 4 elsewhere.

Other Features

The *racial incidence* proved interesting. Of the cases in which race was known, 28 occurred in white subjects and 21 in Negroes. Considering the over-all ratio of white to Negro population as roughly 10 to 1, the anticipated incidence among Negroes would be 10 per cent, whereas it was actually 43 per cent in this series.

Duration of known existence of the tumor preceding excision ranged from one day to 15 years, those in the tongue having been noted and removed somewhat earlier than those in other locations.

Diameter of the tumors varied from 3 to 40 mm.; the extralingual ones, having been removed later, were larger.

Pain was mentioned in the records of only 6 cases. *Tumefaction* was usual, although actual retraction had occurred over several of the lingual neoplasms, the tumors having been manifest by their greater density. To our knowledge there was *no recurrence* of a tumor after excision.

DISCUSSION

Several points favor the concept of neurogenesis of "granular cell myoblastomas". The strongest is the demonstration of concentric masses of granular tumor cells with cores consisting of bundles of axis cylinders and peripheries resembling expanded and frayed nerve sheaths. It is significant that such structures would be clearly demonstrated within or at the margins of the tumors, sometimes extending well beyond, in 33 cases (questionable in 4 more) in our series, totalling 52. Also impressive was the finding of granular tumor cells, singly or in small clumps, within the sheaths of nerve twigs at some distance from the tumors proper. Certain tumors displayed a general pattern reminiscent of peripheral nerve tumors, with the cells arranged in fasciculi and frequently separated by delicate wavy fibrillae. This was particularly striking in a series

of 4 tumors having many features of the conventional neurofibroma, but in which the granular cell components were progressively greater.

A study of "granular cell myoblastomas" that lay within or in immediate juxtaposition to striated muscle failed to disclose conclusive evidence of transition from muscle to granular tumor cells. Pseudotransitions were abundant among the lingual lesions, due to tumor cells overlapping muscle fibers cut slightly tangentially. Bodian preparations revealed differences between tumor cell granules and those due to degeneration of myofibrillae.

We agree with Gray and Gruenfeld that there is no resemblance between human embryonal muscle cells and granular tumor cells. Again, there is no close similarity between the latter and the developing canine muscle cells pictured by Godlewski, from which Abrikossoff originally drew the analogy and coined the term "Myoblastenmyome". *It is interesting to speculate as to what name Abrikossoff would have given these tumors had his first 5 been found in areas devoid of skeletal muscle.*

There is no good evidence for the existence of a tumor derived from or made up of myoblasts, cells that are found only in the embryo. The tumors under discussion appear to arise from nerves. The terms rhabdomyoma and rhabdomyosarcoma should suffice for tumors truly taking origin in skeletal muscle. Thus, it would seem well to discard "myoblastoma" as a name for neoplasms thus far described. We propose the term "granular cell neurofibroma" for the benign tumors heretofore called "granular cell myoblastoma".

CONCLUSION

Histologic study of 52 cases of "granular cell myoblastoma" arising in association with skeletal muscle and elsewhere indicates that these tumors are derived from nerves and not from skeletal muscle.

Acknowledgments. We are indebted to Dr. O. O. Williams, St. Joseph's Hospital, Phoenix, Arizona, and Dr. J. W. Hooker, Memorial Hospital, Wilmington, Delaware, each of whom contributed a case to our series; and to Dr. M. J. Hogue, Department of Anatomy, University of Pennsylvania School of Medicine, for her opinion regarding the dissimilarity of tumor cells and primitive myoblasts.

REFERENCES

1. Abrikossoff, A.: Über Myome, ausgehend von der quergestreiften willkürlichen Muskulatur. Virchows Arch. f. path. Anat., **260:** 215–233, 1926.
2. Abrikossoff, A. I.: Weitere Untersuchungen über Myoblastenmyome. Virchows Arch. f. path. Anat., **280:** 723–740, 1931.
3. Crane, A. R., and Tremblay, R. G.: Myoblastoma (granular cell myoblastoma or myoblastic myoma). Am. J. Path., **21:** 357–375, 1945.
4. Fust, J. A., and Custer, R. P.: Granular cell "myoblastomas" and granular cell "neurofibromas"; separation of neurogenous tumors from the myoblastoma group. Am. J. Path., **24:** 674, 1948.
5. Godlewski, E.: Die Entwicklung des Skelet- und Herzmuskelgewebs der Säugetiere. Arch. f. mikr. Anat., **60:** 111–156, 1902.
6. Gray, S. H., and Gruenfeld, G. E.: Myoblastoma. Am. J. Cancer, **30:** 699–708, 1937.
7. Klemperer, P.: Myoblastoma of striated muscle. Am. J. Cancer, **20:** 324–377, 1934.
8. Klinge, F.: Über die sogennanten unreifen, nicht quergestreiften Myoblastenmyome. Verhandl. d. deutsch. path. Gesellsch., **23:** 376–382, 1928.
9. Roffo, A. H.: Knotige Zungenmyolyse. Ztschr. f. Krebsforsch., **39:** 464–470, 1933.
10. Simon, M. A.: Granular cell myoblastoma (myoblastic myoma, rhabdomyome granulocellulaire). Am. J. Clin. Path., **17:** 302–313, 1947.

AN EVALUATION OF THE USE OF GROUP-SPECIFIC SUBSTANCES IN GROUP O BLOOD TRANSFUSIONS*

OSCAR M. ALTON, M.S., AND WILLIAM R. PLATT, M.D.

From the Departments of Pathology of the Norton Memorial Infirmary and the University of Louisville School of Medicine, Louisville, Kentucky

During World War II Group O donor blood was often used indiscriminately with only occasional hemolytic transfusion reactions in Group A, B, or AB recipients. These reactions presumably were produced by the high titer of anti-A and/or anti-B isoagglutinins in some of the Group O bloods administered. In fact, Ebert and Emerson[1] found that febrile hemolytic reactions accompanied by chills were rarely observed following either single or multiple transfusions of Group O blood into recipients whose blood groups were A, B, or AB. In the course of 265 consecutive transfusions of this type, they observed only 3 reactions (1.1 per cent) accompanied by hemoglobinemia and hyperbilirubinemia. Each of the 3 reactions was associated with an isoagglutinin titer of the blood exceeding 1:500. Ebert and Emerson also found an increase in osmotic fragility of the erythrocytes of the recipients following transfusions of Group O blood and pooled plasma to patients of blood groups other than Group O; this phenomenon being related to the concentration of incompatible isoagglutinins and the frequency of their administration. These reactions were also confirmed by Tisdall *et al.*[3] in an actual clinical experiment. The latter workers produced hemolytic reactions of varying severity by giving transfusions of Group O blood (with an isoagglutinin titer of 400 units or higher) to recipients of other blood groups.

Therefore, in order to facilitate the use of Group O blood as a "universal donor without danger", it was considered advisable to find a rich source of group-specific substances which might reduce the titer of isoantibodies in O blood when added to a pint of O blood either as soon as the blood was collected or before the transfusion was given. Accordingly, Witebsky and Klendshoj[8] in 1940 isolated a carbohydrate preparation (exhibiting B properties) from the gastric juice of human beings. However, the quantity available was negligible so that other sources of blood group-specific substances had to be investigated. Subsequently, the mucous membrane of the horse's stomach was found to contain varying amounts of both A and B substances. In order to obtain sufficiently large amounts of the final preparation (an AB substance), the gastric mucosa, taken from several horses, had to be pooled after the material had previously been digested with pepsin and repeatedly precipitated with alcohol. The final AB product is a whitish powder. Several milligrams of this product are dissolved in sterile physiologic saline and the colorless solution is sealed in ampules.† These preparations have been found to be chemically free of protein and their

* Received for publication, December 22, 1948.

† The authors wish to express their appreciation to Dr. E. J. Teeter of Eli Lilly & Co. and to Dr. William Boger of Sharpe and Dohme, Inc. for their generous supplies of group-specific substances in solution.

failure to sensitize guinea pigs and other animals is subject to rigid specifications set up by a committee of the National Research Council.[7]

Although many investigations have been carried out previously[8] and published by other workers[2, 4, 6, 8, 10, 11] on the use of soluble A and B group-specific substances for addition to Group O blood in order to reduce its isoagglutinin titers to safe levels, it was considered advisable to repeat the experiment on a series of random Group O blood from the standpoint of its value in a small private hospital. Therefore, the following experiment was carried out during 1947 and 1948 in a 155-bed, private hospital whose blood bank facilities were quite limited and whose transfusion reaction rate was 2.34 per cent (1451 transfusions) in 1947 and 1.86 per cent (1501 transfusions) in 1948. It was believed that if the procedure was found to be of value, a similar set-up could be established in comparable hospitals in rural areas, thereby diminishing the number of possibly serious hemolytic transfusion reactions, especially in emergency transfusions when blood of Group A, B, or AB was not immediately available. The following experiment, therefore, attempts to demonstrate:

(a) The efficacy of the addition of isolated blood group-specific substances in suppressing isoagglutination *in vitro*.

(b) The apparent safety and value in its use in emergency transfusions for hemorrhage in civilian life.

MATERIAL AND METHODS

The usual pre-transfusion tests were carried out on the recipient regardless of whether Group O blood or bloods of identical groups were to be used. These tests consisted of determination of the blood group and Rh factor, cross-matching and serologic tests for syphilis. In those cases where Group O blood was to be used as a "universal donor" and when the minor cross-matching (donor's serum and recipient's cells) only showed slight agglutination, the blood was usually given slowly to the recipient. However, if marked agglutination or hemolysis instead of slight agglutination was observed on this minor cross-matching, the blood was considered incompatible and was not used. All Group O donors were accepted by the Norton Memorial Infirmary Laboratory as long as there was no history of allergic phenomena (hay fever, angioneurotic edema), rheumatic fever, tuberculosis, syphilis, malaria, undulant fever or jaundice. In addition, the donor was required to be in good health, have no history of recent loss of blood and have had nothing to eat or drink in the four hours prior to donation. Baxter Transfuso-Vac sets containing 120 ml. of dextrose, sodium citrate and citric acid were used, (each 100 ml. containing 3.30 Gm. of dextrose U.S.P., 1.33 Gm. sodium citrate U.S.P., 0.47 Gm. of citric acid U.S.P.). All donor bottles were chilled to a temperature between 4 C. and 10 C. prior to collection of blood. The blood was administered by venoclysis, using disposable sterile equipment. The same, properly qualified, experienced worker performed all withdrawals. This same worker was also responsible for proper labeling and identification of the blood as to group, Rh factor, serology, name of donor and date of withdrawal. The top of the container was then covered with sterile

gauze and adhesive tape and returned to the laboratory refrigerator to be used within 7 to 10 days before possible use in pooling of plasma.

Immediately before transfusion the Group O blood was gently inverted several times and 10 ml. of its contents were withdrawn into a sterile 20 ml. syringe and subsequently placed in a labeled test tube. Using another syringe and needle, the contents of one ampule (approximately 10 ml., enough to neutralize the A and B isoagglutinins usually present in 500 ml. of blood) of A and B Group-specific substances (of Witebsky) were injected through a sterilized rubber stopper into the Group O blood bottle. The latter was then gently mixed by inversion for approximately three to five minutes after which a second 10 ml. portion of the prepared blood was withdrawn and placed in another labeled test tube.

The titer of anti-A and anti-B agglutinins in the donor's Group O blood was then determined in the following manner: A specimen of donor's blood taken before and a specimen taken after the addition of Witebsky's group substance were centrifuged and the plasma pipetted from each. Serial plasma dilutions ranging from 1:2 to 1:1024 were then prepared, using sterile physiologic saline as the diluent. Equal parts (0.2 ml.) of the serially diluted plasma and a 2 per cent isotonic saline suspension of washed Group A red blood cells were mixed in Kahn tubes and read after standing 45 minutes at room temperature. A similar set-up was prepared using a 2 per cent saline suspension of washed Group B erythrocytes. Each completed protocol consisted of 4 rows of suspensions in Kahn tubes, 11 tubes in each row. In reading the results the highest dilution of plasma showing agglutination (using the hand lens) was considered the titer of the corresponding agglutinins present.

RESULTS

A total of 91 Group O blood transfusions were given to recipients of Groups A, B and AB. The titers of anti-A and anti-B agglutinins were determined before and after the addition of Witebsky's Group-specific substances. The highest anti-A agglutinin titer obtained was 1024 and the lowest was 2. The sum total titer in this series was 13,424; this was reduced to 624 after the addition of the group-specific solutions. The average mean titer of anti-A agglutinins was 148; this was reduced to 7 after Witebsky's solution was added, a reduction of 95.4 per cent in the mean titer of this anti-A group. In 41 cases the anti-A agglutinins were completely neutralized; the average titer in the remaining 50 was 12.4. The highest anti-B agglutinin titer obtained was 512 and the lowest 0. The sum total titer in this series was 7476; this was reduced to 440 after the addition of group-specific solutions. The average mean titer of anti-B agglutinin was 82.1. This was reduced to 4.8 after Witebsky's solution was added, a reduction of 94.2 per cent in the mean titer of the anti-B group. In 57 cases the anti-B agglutinins were completely neutralized; the average titer in the remaining 34 was 13.0. A presentation of the isoagglutinin variation in the entire series is given in Figure 1.

Five mild so-called "transfusion reactions" are noted in the entire series of 91 cases. These are presented below in detail and are abstracted in Table 1.

Mrs. M. K. (Case 3), age 20, blood Group A, Rh-positive, was admitted to the hospital with a diagnosis of anemia of pregnancy with associated bleeding. A 500 ml. transfusion of Group O blood, containing 10 ml. of Witebsky's group-specific substances in solution was given without any immediate subjective or objective reaction. The titers of anti-A and anti-B agglutinins in the donor's blood, were 16 and 32, respectively; after addition of the group-specific sub-

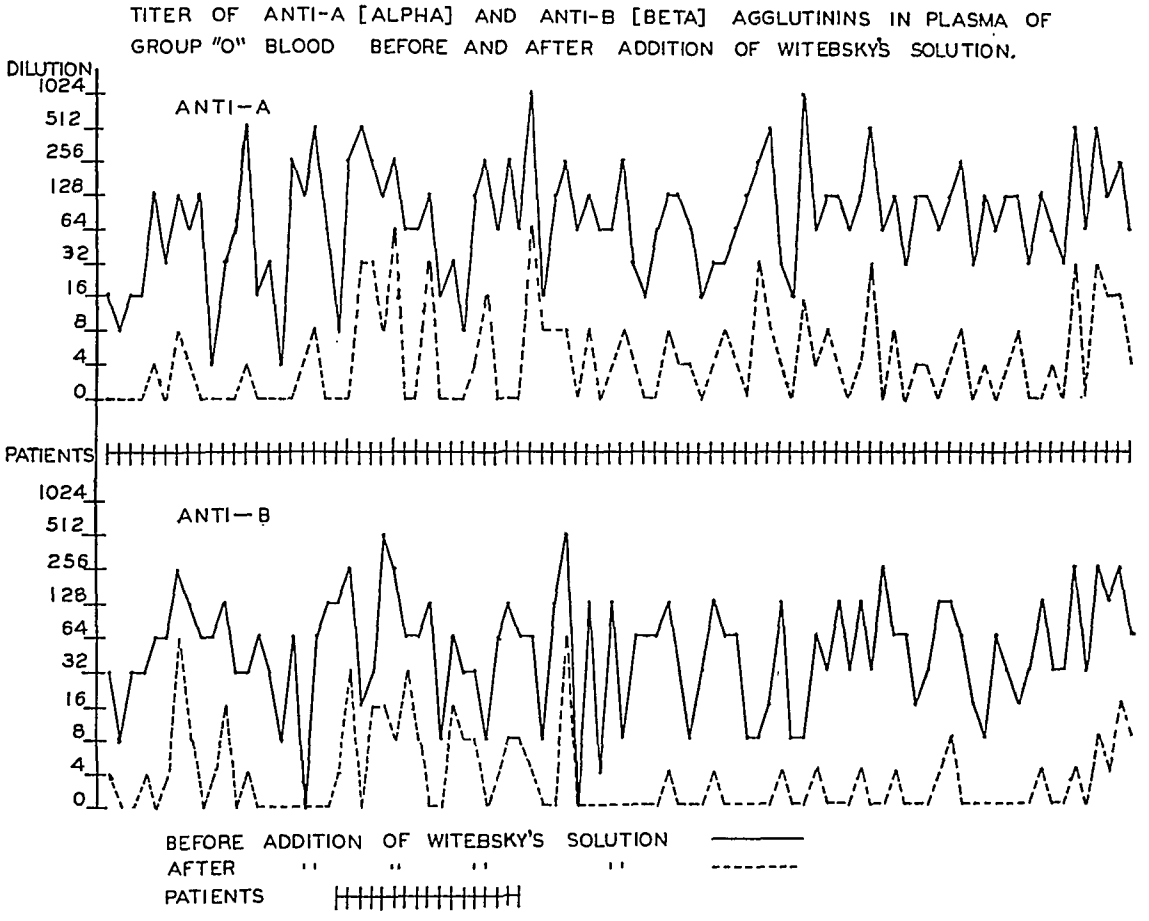


FIG. 1

stances, complete neutralization of the isoagglutinins was observed. The patient was discharged from the hospital the day after transfusion. She reported to her doctor that she had a "reaction" after she reached home. The details of this "reaction" were not available.

Mr. T. G. (Case 9), age 71, Group A, Rh-positive, was admitted to the hospital with a diagnosis of carcinoma of the liver. He received a total of 5 transfusions approximately four days apart. Group A, Rh-positive blood was used for the first 3; Group O, Rh-positive blood containing 10 ml. of Witebsky's solution for the fourth, and Group A, Rh-positive blood for the fifth transfusion. After each transfusion the patient had a rise in temperature to 99.8, 100.2, 100,

100.8, and 100 F., respectively. There were no chills and no medication was given. Before neutralization with group-specific substances the agglutinin titer in the fourth transfusion was 64, anti-A and 64, anti-B; afterwards the anti-A was 4 and the anti-B, 0.

Mrs. M. B. (Case 10), age 51, Group A, Rh-negative, was admitted to the hospital with a diagnosis of cystocele, rectocele, chronic cervicitis and fibrosis

TABLE 1

SUMMARY OF DATA IN 5 PATIENTS WITH REACTIONS

NO.	PATIENT	AGE	BLOOD GROUP AND Rh TYPE	EFFECT ON TITER OF ADDING WITEBSKY'S SOLUTION				TYPE OF REACTION	DURATION	MEDICATION	REMARKS
				Anti-A Titer		Anti-B Titer					
				Before	After	Before	After				
3	Mrs. M. K.	20	A Rh+	16	0	32	0	Not stated	?	None	Patient in hospital 24 hrs. Reaction (?) at home
9	Mr. T. G.	71	A Rh+	64	4	64	0	Slight fever	2 hrs.	None	5 transfusions, 4 with Rh+ blood. Fever after each.
10	Mrs. M. B.	51	A Rh−	128	4	64	2	Fever, chill, sweating	15 min.	External heat	Discomfort in bladder
54	Mrs. M. H.	36	B Rh+	128	2	64	2	Fever, itching, chill	24 hrs.	External heat	Reaction from the catheterization (?)
55	Mr. T. S.	63	A Rh+	64	0	64	0	Slight fever, chill	2 hrs.	None	Mild reaction

uteri. Two transfusions were given; 500 ml. on September 24th using Group A, Rh-negative blood (the anti-A titer was 128 before the addition of group-specific substances, and 4 afterwards; the anti-B titer was 64 before and 2 afterwards). Five hours after the second transfusion the patient stated she felt "uncomfortable in the bladder", perspired and then had a 15-minute chill. External heat was applied and in approximately 15 to 20 minutes the patient felt much better.

The blood had been started at 2:30 P.M.; at 4 P.M. the temperature was 102.6 F., pulse rate was 100 and respiratory rate was 30; at 6 P.M. the temperature was 101.6 F., pulse rate was 96, respiratory rate was 28; at 7 P.M. (the time of the chills) the temperature was 103.6 F., pulse rate was 100, respiratory rate was 32. Within 24 hours the temperature, pulse and respiratory rates were all normal.

Mrs. M. H. (Case 54), age 36, Group B, Rh-positive, was admitted to the hospital for surgery with a diagnosis of "old vaginal and cervical laceration and Graves' disease". On December 13th (temperature 99 F. and two days post-operative), the patient received 500 ml. of Group O, Rh-positive blood. The anti-A titer was 128, anti-B was 64; after Witebsky's solution had been added the titers were anti-A, 2 and anti-B, 2. The transfusion was completed at 9:30 A.M. At 10:50 A.M., the patient complained of "itching all over". At 8 P.M. the temperature was 102 F.; 24 hours later (on December 14) it was 104 F. and external heat was applied. On December 15, it had dropped to 98 F. A progress note on the chart dated December 14 stated that a "non-functioning catheter" was removed from the bladder. The chill associated with the transfusion may have been due to bladder irritation.

Mr. T. S. (Case 55), Group A, Rh-positive, was admitted to the hospital on December 11 for cystostomy with a diagnosis of prostatism. On December 13 at 8:45 A.M., the patient received 500 ml. of Group O blood (anti-A and anti-B titers were each 64 both before and after Witebsky's solution was added). The transfusion was finished at 1 P.M. At 2:30 P.M. the patient had chilly sensations, temperature was 99.2 F., the pulse rate was 104; at 4 P.M. the temperature was 102.4 F., the pulse rate 125, the respiratory rate 22. Eighteen hours later the temperature was normal. No medication was given.

DISCUSSION

As can be observed from the above collection of data, universal donor Group O blood modified by group-specific substances may be given with the minimum possibility of post-transfusion reaction. In fact, no serious hemolytic sequelae (*i.e.*, no hemoglobinemia or hyperbilirubinemia) were observed in any of the 91 cases described in this series. This was most gratifying since the crucial test usually lies in the high anti-A and anti-B isoagglutinin titer Group O donors. Of the 5 "so-called" reactions observed, only 4 had possible symptoms which could place them in this category. The symptoms in Case 3 were vague and undetermined. In the remaining 4 cases, the reactions were mild and resembled reactions due to causes other than those associated with the usual antigen-antibody transfusion type. For example, the fact that Cases 9 and 10 and Cases 54 and 55 occurred in succession makes one suspicious of possible pyrogens, faulty tubing, Rh sub-groups and even A sub-groups—although the latter isoagglutinins are thought to be neutralized by the group-specific substance solutions.[7] Other workers in the field believe that Witebsky's solution has definite advantages in certain specific instances. According to the work of Tisdall *et al.*,[4] it is of prime importance in military medicine in the treatment of combat casualties, *i.e.*, the routine addition of A and B substances to Group O blood would render any

Group O blood perfectly safe for use as universal donor blood for military purposes. Its use in the immediate treatment of emergency cases of hemorrhage in civilian life and in hospitals without proper blood bank facilities has certain limitations. For example, male recipients receiving multiple transfusions of Group O blood (so modified) may show a definite rise in anti-B agglutinin titer (as much as sixfold in some instances). According to Tisdall and his group[4], this does not produce any serious sequelae. However, Wiener[5] recently has described a case of erythroblastosis fetalis which he believes was caused by the action of anti-A isoagglutinins of a Group-O mother on the Group-A red cells of a fetus *in utero*. According to him, this is more likely to occur when there is a high titer of isoagglutinins in the maternal circulation. Other workers in this field believe that the evidence which Wiener presents for his conclusions in this case is weak. Furthermore, they do not wholly agree with his views that there is a minimal but definite danger in stimulating increased antibody formation in female patients potentially capable of child-bearing if one uses Group O blood modified by the addition of group-specific substances. Strong support for their conclusion is based on the fact that pooled plasma also contains dissolved group-specific substances. Of course, pooled plasma has been used on a large scale all over the United States but so far no observation has been reported which would lead to the belief that immunization with the group-specific substances has caused erythroblastosis in infants. Working along different channels, Witebsky[7] has shown that the addition of blood group-specific substances results not only in the reduction of the end titer but also in the reduction of quality of agglutination in the remaining dilutions which still show some agglutination. He also demonstrated that the isohemolysis is completely inhibited by the addition of blood group substances. Still another practical application of the purified blood group-specific substances consists in the elimination of anti-A and anti-B agglutinins in anti-Rh serums used for the determination of the presence of the Rh factor. In order to allow the anti-Rh antibody to act specifically against Rh-positive cells, the isoagglutinins anti-A and anti-B have to be completely neutralized, *i.e.*, the titer reduced to zero. Therefore, in order to be sure that no trace of anti-A and anti-B agglutinins remain (which could result in erroneous designation of the Rh factor), a surplus of group-specific substances A and B should be added. A final application of these A and B substances is in the production of potent blood-grouping test serums resulting in fewer mistakes in routine blood group determinations.[11] The test serums so produced show a 10 to 100-fold increase in the original agglutinin titer with frequent shortening of the time of agglutination. A_2, A_2B and even A_3 cells are strongly agglutinated by the serum of patients treated with blood group-specific substances.[7]

SUMMARY

1. A series of 91 transfusions using Group O blood (Witebsky's group-specific substances added) were given to recipients of blood groups A, B and AB.

2. An evaluation of the efficacy of this solution shows complete neutralization of anti-A isoagglutinins in 41 of the 91 Group O bloods, an over-all reduction of

95.4 per cent in isoantibody titer. The anti-B isoagglutinins were completely neutralized in 57, a reduction of 94.2 per cent in antibody titer.

3. Five mild "so-called" reactions were noted. These are discussed. No evidence of hemoglobinemia or hyperbilirubinemia was noted.

4. Group O blood, containing Witebsky's solution, may be used efficaciously in small hospitals that have limited blood bank facilities.

REFERENCES

1. Ebert, R. V., and Emerson, C. P., Jr.: A clinical study of transfusion reactions; hemolytic effect of group O blood and pooled plasma containing incompatible isoagglutinins. J. Clin. Investigation, **25:** 627–638, 1946.
2. Klendshoj, N. C., and Witebsky, E.: Tranfusion of O blood conditioned by addition of blood group specific substances. J. A. M. A., **128:** 1091–1093, 1945.
3. Tisdall, L. H., Garland, D. M., Szanto, P. B., Hand, A. M., and Bonnett, J. C.: The effects of the transfusion of group O blood of high isoagglutinin titer into recipients of other blood groups. Am. J. Clin. Path., **16:** 193–206, 1946.
4. Tisdall, L. H., Garland, D. M., and Wiener, A. S.: A critical analysis of the value of the addition of A and B group specific substances to group O blood for use as a universal donor blood. J. Lab. and Clin. Med., **31:** 437–443, 1946.
5. Wiener, A. S.: Rh factors in clinical medicine. J. Lab. and Clin. Med., **30:** 957–976, 1945.
6. Wiener, A. S., and Pennell, S. S.: The life-saving power of "safe" universal donor blood in exsanguinating hemorrhage. Am. J. M. Sc., **204:** 1–4, 1942.
7. Witebsky, E.: Isolation and purification of blood group A and B substances; their use in conditioning universal donor blood, in neutralizing anti-Rh sera and in the production of potent grouping sera. Ann. New York Acad. Sc., **46:** 887–898, 1946.
8. Witebsky, E., and Klendshoj, N. C.: The isolation of the blood group specific B substance. J. Exper. Med., **72:** 663–667, 1940.
9. Witebsky, E., Klendshoj, N. C., and McNeil, C.: Potent typing sera produced by treatment of donors with isolated blood group specific substances. Proc. Soc. Exper. Biol. and Med., **55:** 167–170, 1944.
10. Witebsky, E., Klendshoj, N. C., and Swanson, P.: Preparation and transfusion of safe universal blood. J. A. M. A., **116:** 2654–2656, 1941.
11. Witebsky, E., Klendshoj, N. C., and Swanson, P.: Neutralization of isoagglutinins anti-A and anti-B in O blood by means of the addition of the isolated blood group specific substances. In: Blood Substitutes and Blood Transfusions. Edited by Mudd, S., and Thalhimer, W. Springfield, Ill.: Charles C Thomas, pp. 327–336, 1942.

THE DIFFUSELY ENLARGED UTERUS*

K. M. TRUEMNER, M.D., AND D. H. KAUMP, M.D.

From the Departments of Pathology, Providence Hospital, and Wayne University College of Medicine, Detroit, Michigan

During the past century the clinical picture of abdominal pain, menorrhagia, and leukorrhea associated with the pathologic picture of a diffusely enlarged uterus has been attributed, either primarily or in combination, to subinvolution,[16, 19, 23] inflammation with fibrosis of the myometrium,[15, 24, 25] primary vascular changes,[6, 9, 10] muscular hypertrophy,[8, 13, 27] and endocrine dysfunction.[1, 4]

This study is based on the examination of 64 nontumor-bearing uteri of which 52 were diffusely enlarged, 6 were atrophic, 1 was obtained at necropsy from a pregnant woman at term, and 5 were normal uteri which served as controls. We regarded uteri as enlarged if their dimensions, exclusive of the cervix,[27] exceeded a length of 5 cm., a width of 4.4 cm., and a thickness of 2.2 cm. and if the maximum thickness of the myometrium exceeded 1.5 cm.

Both clinical and pathologic surveys were made. On each patient from whom a uterus was obtained a detailed clinical abstract was prepared which contained information as to the age, number of deliveries, menstrual history, gynecologic complaints, pertinent physical and laboratory findings, and associated medical or surgical lesions. The pathologic abstracts included gross observations as to the size of the uterus, microscopic findings, and maceration-extraction studies of the myometrial wall.

CLINICAL SUMMARY

For purposes of comparison we divided our 52 patients with enlarged uteri into a group of 24 with a history of abnormally increased menstruation and a group of 28 without abnormally increased menstruation. These groups are referred to as menorrhagic and non-menorrhagic in the balance of this paper. In each group there was one nulliparous patient with an enlarged uterus.

In both groups of patients, the average age (menorrhagic, 41 years; non-menorrhagic, 42 years) and the incidence of such clinical symptoms as leukorrhea, low back pain, pelvic pain and menstrual irregularity were similar. In spite of the history of increased menstrual flow in the menorrhagic group it is notable that only 5 patients had blood hemoglobin values below 11.5 Gm. per 100 ml. of blood (Sanford-Sheard, sodium carbonate method) at the time of their admission to the hospital. The number of deliveries ranged from none to 7 in each group.

* Abridgement of thesis submitted by K. M. Truemner to the Faculty of the Graduate School of Wayne University College of Medicine in partial fulfillment of the requirements for the degree of Master of Science in Pathology.

Received for publication, September 27, 1948.

GROSS PATHOLOGIC SUMMARY

On cut section the color of the myometrium varied from pale yellow to yellowish pink. The consistency was uniformly increased and firm and in occasional cases the vascular channels were prominent but characteristically the myometrium was smooth throughout. Significant degrees of edema and fibrosis of the outer third of the myometrium were present equally in both groups and were usually in association with chronic pelvis inflammatory disease, retroversion and prolapse.

In considering the size of the uterus and the myometrial thickness in relation to the age of the patients from both groups, the diffusely enlarged uteri, as examined in the laboratory, were found almost entirely in women between the ages of 35 and 50 years. This is the period during which the menopause is reached. Nineteen of the 24 patients with menorrhagia and 18 of the 28 patients without menorrhagia were in this age group.

Small endometrial polyps were found in 2 cases of the menorrhagic group but otherwise the endometrium was grossly similar in both groups. Cortical ovarian retention cysts, cystic cervicitis, and evidence of chronic inflammation in the fallopian tubes were found equally in both groups. Simple serous cystomas of the ovary were found in two instances.

MICROSCOPIC STUDY

For histologic studies we used formalin-fixed sections of full thickness blocks of the uterine wall and of associated structures, stained with hematoxylin-eosin, Masson's trichrome and Weigert-van Gieson stains. In the microscopic sections the endometrium was studied for evidences of inflammation and of endocrine imbalance. Extravascular collections of leukocytes were considered to be evidence of inflammation, and cystic change, glomerular forms, sub-basilar glands and diphasic changes were considered as evidence of endocrine imbalance. The submucosal, vascular, and subserosal myometrial areas were examined for the amount, type, and distribution of connective tissue and its relation to muscle fibers. The connective tissue was recorded as normal, slightly increased, or significantly increased. The arteries, veins, and lymphatics in each myometrial layer were similarly analyzed. The muscle was studied for evidence of mitotic activity.

The microscopic findings are summarized in Table 1.

Twenty-seven of the diffusely enlarged uteri in this series had an essentially normal histologic architecture (Fig. 1) and 25 had a significant degree of connective tissue increase. The increase in connective tissue was chiefly of a fibrillar form and had a perivascular distribution with radiating strands between and separating the muscle bundles. This increase in connective tissue assumed two fairly distinct forms of distribution, a diffuse subserosal interstitial increase (Fig. 2; 21 cases) and a diffuse increase noted in all three layers of the myometrium (4 cases). The subserosal connective tissue increase was found in association with inflammatory lesions of the pelvic viscera or in those patients with clinical evidence of prolapse. In those found in association with chronic

suppurative salpingo-oophoritis, there were organized fibrous exudates on the uterine serosa but in no case was there evidence of extension of any inflammatory process into the inner portions of the myometrial wall. In those with clinical evidences of prolapse and retroversion there was microscopic evidence of venous and lymphatic dilatation (Fig. 3). In many sections large masses of collagen surrounded these vascular structures and extended out into the interstitial tissue of the myometrium. In the 4 uteri with diffuse fibrosis this change was prominent in the outer third of the myometrium but with no evidence of either associated adnexal inflammation or of vascular stasis. Baker has pointed out that in the uterus an increase in age is characterized by replacement of muscle with connective tissue, beginning with the serosa and progressing centrally. In rats the aging process of the uterus is characterized by progressive deposition and condensation of connective tissue.[5,28,29] We considered that in these cases an early aging process had begun. This concept was further supported by our own studies of senile uteri (6 cases) in which there was progressive fibrosis beginning in the subserosal zone.

TABLE 1

MICROSCOPIC FINDINGS IN DIFFUSE ENLARGEMENT OF THE UTERUS

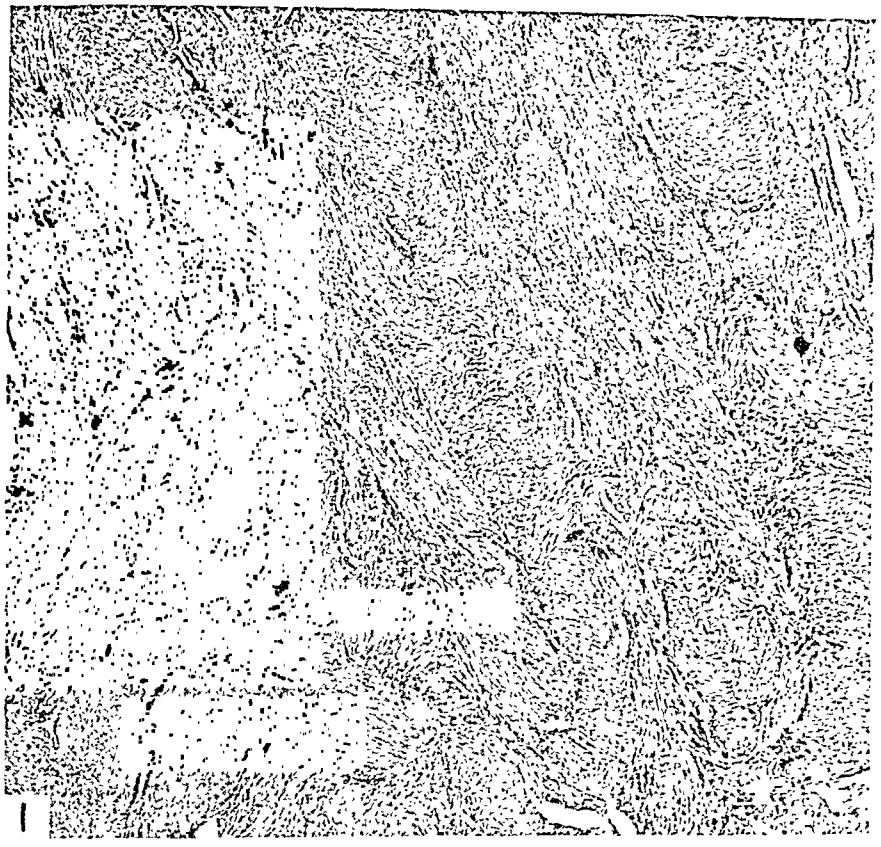
MICROSCOPIC FINDINGS	MENORRHAGIC GROUP (24 CASES)	NONMENORRHAGIC GROUP (28 CASES)
Endometrial alterations	11	7
Chronic inflammation	0	0
Subserosal connective tissue increase	8	13
Diffuse connective tissue increase	1	3
Marked changes resulting from pregnancy	2	4
Mitotic forms	0	0

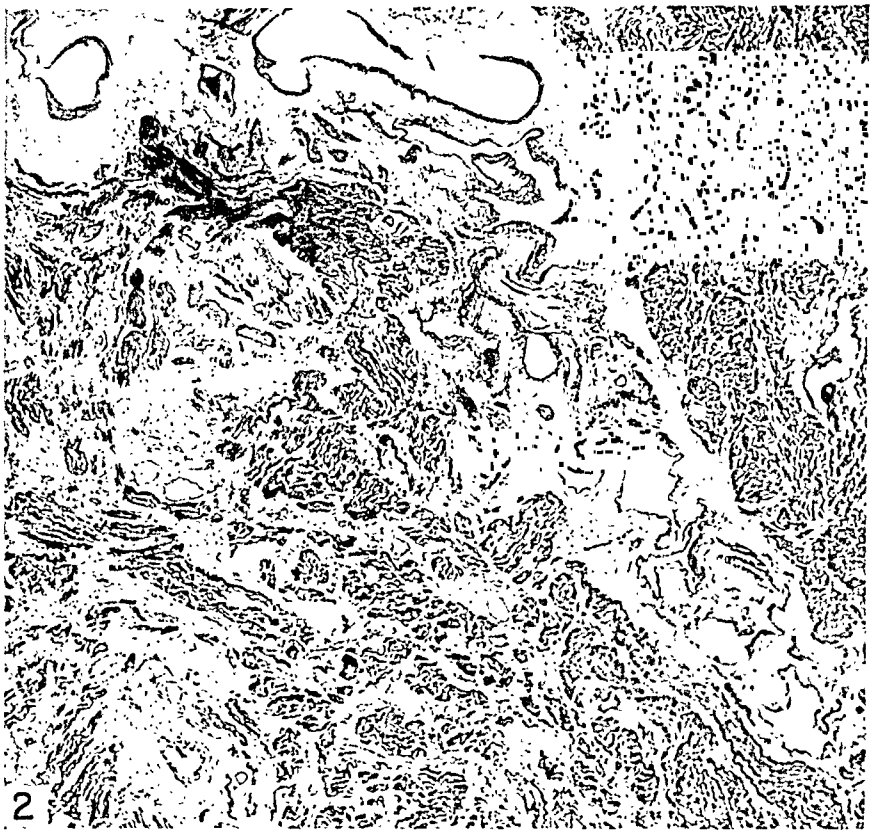
Consequently, we feel that the term "chronic metritis" has little if any place in the nomenclature or in the description of the diffusely enlarged uterus. "Fibrosis" of the uterus may be a term worthy of retention if it is used in the restricted sense of implying a process secondary to adnexal inflammation, to stasic change, or to the aging process.

The changes resulting from parity were best demonstrated by the Weigert-van Gieson stains. These changes consisted of an increase in elastic tissue masses separating the medial muscle fibers and lying between the media and adventitia (Fig. 4). From occasional adventitial masses there were delicate elastic strands extending into the adjacent interstitial tissue. There was also medial collagen deposition with increased adventitial collagen paralleling the elastic tissue changes. There was no definite correlation between the number of pregnancies and the histologic residuals except that there was a general tendency

FIG. 1. From a diffusely enlarged uterus with normal connective tissue and muscle proportions in a 49 year old multipara. Average diameter of muscle fibers 5.94 microns. Masson's stain. × 40.

FIG. 2. Severe subserosal fibrosis and stasis changes in a 38 year old parous woman with chronic suppurative salpingo-oophoritis. Masson's stain. × 40.





FIGS. 1-2

for the most marked changes to occur in those women who had had most pregnancies. Venous and lymphatic dilatation was prominent in occasional sections and suggested the possibility that mechanical stasis may be a factor in the failure of normal resolution.

The changes induced by pregnancy apparently have little relationship to menorrhagia when we consider that they were more severe and frequent in the uteri from nonmenorrhagic than from menorrhagic women. We do believe that subinvolution is a pathologic term worthy of retention when it is applied to those uteri in which special elastic stains reveal a definite exaggeration of residual changes of pregnancy, as described in the preceding paragraph.

In the group of 52 uteri there were 8 in which there was severe vascular medial and adventitial collagen deposition and varying degrees of subintimal thickening. These 8 uteri came from patients with severe retroversion (2 cases), cardiac decompensation (1 case), prolapse of the uterus (4 cases), and previous radium insertion (1 case). There was no difference in the character and distribution of the vascular changes in the menorrhagic and in the nonmenorrhagic groups. In no case was the bulk of the vascular structures increased sufficiently to produce an increase in the size of the uterus. We can attribute little importance to vascular changes as a primary factor to either increased size of the uterus or to the tendency to menorrhagia.

Quantitative estimation of connective tissue in the uterine wall was obtained by determination of weight before and after extraction with sodium hydroxide.[26] The amount was expressed as per cent of residual weight and represents the per cent of connective tissue in a given portion of myometrium. This was controlled by rehydration of the tissue and microscopic study to demonstrate the absence of all muscle tissue. Duplicate determinations were made in sufficient numbers from the same uterus to demonstrate close agreement.

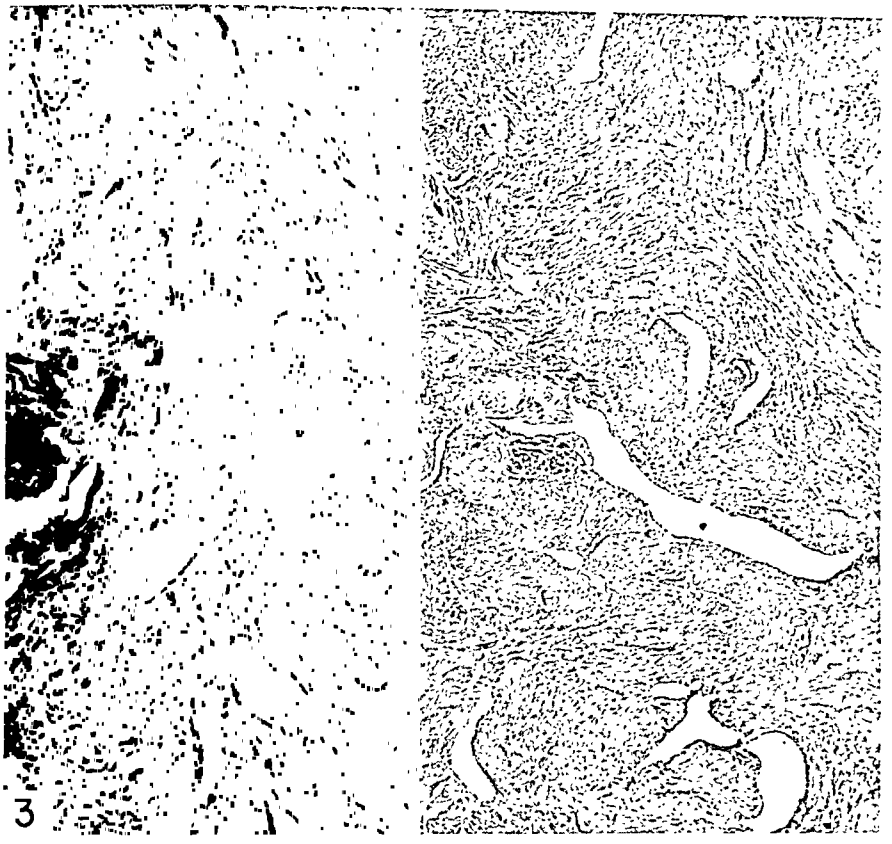
Our series of connective tissue extractions is of interest because of the general agreement with the gross and microscopic observations. The percentage of connective tissue extracted in this series is listed in Table 2.

From our connective tissue extraction studies we found the higher percentages of connective tissue present in those uteri in which the basic architecture was altered by an increase in collagenous connective tissue either on the basis of adnexal inflammation or stasis.

The size of muscle fibers was measured in the following manner on Masson-stained sections: Using a fixed rigid mounting, the sections were projected with an oil-immersion lens onto a 45 degree angle plane mirror and reflected to the surface of smooth bond paper. The measurements were set up with a 100-micron micrometer scale. For measurement we selected only cells roughly circular or ovoid in cross sections and in which the nucleus was central. Fibers so selected represent the widest possible area and should be more accurate than

FIG. 3. Venous and lymphatic dilatation with moderate fibrosis in a 42 year old multipara with severe retroversion of the uterus. Masson's stain. × 40.

FIG. 4. Classical residual changes of pregnancy. Patient was a 57 year old, para 7. Myometrium 2.5 cm. in thickness. Weigert-van Gieson stain. × 40.





FIGS. 3–4

the cross sectional measurements of longitudinal cells previously reported.[22,27] At least 20 fibers from three different locations were traced, two diameters of each cell were measured, averaged and the cross-sectional areas expressed as square microns.

The results of cross sectional measurements of the muscle fibers are presented in Table 3.

In this study we found an increase in the average muscle fiber diameter of a sufficient degree to cause an actual increase in the size of the uterus. We found no hyperplasia or undue mitotic activity in our cases although mitoses have been reported.[11] The increase in muscle diameter was apparent, not only in

TABLE 2

CONNECTIVE TISSUE EXTRACTION

(In per cent as residual weight)

CASE	MENORRHAGIC GROUP	CASE	NONMENORRHAGIC GROUP
1	10	1	8.7
2	8.3	2	6.5
3	6.9	3	6.2
4	6.1		
5	5.8		

TABLE 3

MUSCLE DIAMETERS

(In Microns)

GROUP	CASES	RANGE	AVERAGE	PER CENT ABOVE NORMAL*
Normal	5		5.30	0
Menorrhagic	19	5.38-6.07	5.82	22
Nonmenorrhagic	26	4.94-6.14	5.62	14

* Percentage above normal is based on computed cross-sectional areas.

cases with normal histologic architecture but also in those uteri in which there were varying degrees of fibrosis due to the secondary factors already described.

Simple myometrial hypertrophy has been defined as a condition found only in nulliparous uteri.[7,14] It is our opinion that this is not the case and that parous uteri also may have myometrial hypertrophy during the menopausal period. We do not believe that this is on a work basis as previously suggested,[18,19,20,21] inasmuch as in our series no significant incidence of truly hyperplastic endometrium was encountered. We believe that there is a moderate but definite muscle-fiber hypertrophy as the primary underlying mechanism of uterine enlargement in the menopausal and premenopausal uterus.

There is some evidence which may explain the muscle-cell hypertrophy. In our studies of the endometrium, evidence of endocrine dysfunction was present in 18 instances consisting of cystic change, glomerular forms, sub-basilar glands, and diphasic changes.

It is quite well established that the basis of menopausal symptoms lies in either a qualitative or quantitative alteration, in estrogen-progestin balance and in the estrogen-androgen ratio. That the uterus is a receptor organ of endocrine metabolism was reported by Heller who found that estrone and estrogenic substance was increased twenty times in potency by incubation with minced uterine tissue. Spleen, heart and lung, and kidney to a lesser extent, affected an increase in potency. Heller suggested that these organs caused an enzymatic reduction of the estrone to the more potent estradiol. It was also demonstrated that liver tissue, unless inhibited by sodium cyanide, has the ability to destroy and inactivate estradiol. In this connection it is of interest that administration of vitamin B complex plus crude liver to women, postpartum will accelerate and enhance the process of uterine involution.[3] In rats, liver is able to inactivate estrone even after exclusion of the reticulo-endothelial system, thus indicating that the liver cell itself must contain the inactivating factor.[30]

In castrated female rats a definite uterine hypertrophy with an increase in length and diameter of the uterine body and cornuae follows prolonged administration of subcutaneous estrone. In addition to simple muscular hypertrophy there is actual hyperplasia of muscle cells.[2]

The uterine size progressively increases during sexual life until true atrophy begins with the completion of the menopause. This phenomenon, unrelated to parity, is attributed to the periodic estrogenic stimulation of the menstrual cycle.[17] Relative or actual excess of estrogen is suggested by the slight difference in the degree of uterine-fiber hypertrophy as demonstrated by the slightly increased cross-sectional area of fibers in menorrhagic women.

It is our opinion that there is a basic progressive enlargement of the uterus in the premenopausal and menopausal age which arises from an absolute or relative excess of estrogen and which may be augmented and modified by such factors as subinvolution, adnexal inflammation, and congestive and stasic changes with the production of varying degrees of fibrosis. At the conclusion of the menopause and at the time of withdrawal of excessive estrogenic stimulation, true senile atrophy begins with a replacement of muscle by collagen, beginning in the subserosal zone. We feel that further strong support would be lent to this concept if it were possible to demonstrate an abnormal estrogen-androgen ratio either quantitatively or qualitatively in women of this age group with enlargement of the uterus. If this could be demonstrated our concept of enlargement of the uterus would rest on a complete and firm foundation.

CONCLUSIONS

There are no apparent histologic changes in the diffusely enlarged uteri of our group to suggest a primary uterine factor for the menorrhagia of women at the menopausal and premenopausal periods. We believe that the basic cause of enlargement of the uterus is a diffuse hypertrophy of muscle fibers, which is probably secondary to abnormal endocrinal stimulation. The connective tissue increase is proportional to this hypertrophy and the histologic architecture is essentially normal.

In certain instances this basic histologic pattern is altered by fibrosis and by the changes of subinvolution. Special stains are frequently necessary to identify the character of these alterations and should be used in the study of this type of uterus.

We suggest the term "menopausal myometrial hypertrophy" for those uteri that have an essentially normal histologic architecture but may be modified by the changes of subinvolution, of stasis, of adjacent inflammation and of aging.

REFERENCES

1. Baker, A. B.: Fibrosis uteri. Am. J. Path., **9**: 369–380, 1933.
2. Barks, O. L., and Overholser, M. D.: Hyperplasia and hypertrophy of uterine musculature in ovariectomized rats following estrone injections. Anat. Rec., **70**: 401–411, 1938.
3. Biskind, L. H., and Biskind, M. S.: Accelerated post-partum involution of uterus with vitamin B complex therapy. West. J. Surg., **52**: 266–270, 1944.
4. Brennecke, Dr.: Zur Aetiologie der "Endometritis fungosa", spezial der "chronischen hyperplasirende Endometritis Olshausen's". Arch. f. Gynäk., **20**: 455–486, 1882.
5. Burack, E., Wolfe, J. M., Lansing, W., and Wright, A. W.: Effect of age upon connective tissue of uterus, cervix and vagina of the rat. Cancer Research. **1**: 227–235, 1941.
6. Cholmogoroff, S.: Sklerose der Uterinarterien. Monatschr. f. Geburtsh. u. Gynäk., **11**: 692–699, 1900.
7. Crossen, H. S., and Crossen, R. J.: Diseases of Women. Ed. 7. St. Louis: The C. V. Mosby Co., 1939.
8. Curtis, A. H.: Hypertrophy of uterus. Am. J. Obst. and Gynec., **50**: 748–752, 1945.
9. Findley, P.: Arteriosclerosis of the uterus as a causal factor in uterine hemorrhage. Tr. Am. Gynec. Soc., **30**: 399–425, 1905.
10. Gardner, W., and Goodall, J. R.: Chronic metritis and arteriosclerotic uterus. Brit. M. J., **2**: 1176–1188, 1906.
11. Hartz, P. H.: Proliferation of muscle cells in myometrium of non-pregnant uterus. Arch. Path., **39**: 323–324, 1945.
12. Heller, C. G.: Metabolism of the estrogens, effect of liver and uterus upon estrone, estradiol and estriol. Endocrinology, **26**: 619–630, 1940.
13. Klebs, E.: Handbuch der pathologischen Anatomie. Ed. 4. Berlin: August Herschemvold, pp. 878–879, 1870.
14. Novak, E.: Gynecological and Obstetrical Pathology. Philadelphia: W. B. Saunders Co., pp. 180–186, 1947.
15. Olshausen, R.: Über chronischen, hyperplasirende Endometritis des Corpus uteri. Arch. f. Gynäk., **8**: 97–133, 1875.
16. Pankow, O.: Die Metropathia hemorrhagica, ein Beitrag zur Lehre von der chronischen Metritis und Endometritis. Ztschr. f. Geburtsh. u. Gynäk., **65**: 336–387, 1909.
17. Piersol, G.: Human Anatomy. Philadelphia: J. B. Lippincott and Co., 2011 pp., 1930.
18. Schwarz, O. H.: Pathology of chronic metritis and chronic subinvolution. Am. J. Obst. and Diseases of Children, **79**: 63–93, 1919.
19. Schwarz, O. H.: Chronic subinvolution, chronic metritis and hypertrophy of uterus. Am. J. Surg., **48**: 321–325, 1940.
20. Shaw, F.: Subdivision of chronic metritis. J. Obst. Gynaec. Brit. Emp., **26**: 73–82, 1914.
21. Shaw, F.: Chronic metritis and allied conditions, in New System of Gynaecology. Vol. 2. Toronto: Macmillan Co., Ltd., pp. 117–151, 1917.
22. Shoemaker, R., and Kahler, J. E.: Fibrosis uteri. Arch. Path., **44**: 621–627, 1947.
23. Szasz-Schwartz, H.: Recherches sur les altérations séniles des vaisseaux sanguins et sur le tissu élastique de l'utérus. Rev. de Gynec., **7**: 593–626, 1903.
24. Theilhaber, A., and Meier, A.: Die Variationen im Bau des Mesometrium und deren Einfluss auf die Entstehung von Menorrhagien und von Fluor. Arch. f. Gynäk., **66**: 1–48, 1902.
25. Von Scanzoni, F. W.: A Practical Treatise on the Diseases of the Sexual Organs of Women. New York: B. M. DeWitt, 95 pp., 1861.

26. WARREN, S., AND WAHL, P. N.: Quantitative estimation of the fibrous tissue in pathologic livers. Arch. Path., 44: 563–570, 1947.
27. WILLIAMS, J. T., AND KINNEY, T. D.: Myometrial hypertrophy (so-called fibrosis uteri). Am. J. Obst. and Gynec., 47: 380–388, 1944.
28. WOLFE, J. M., BURACK, E., LANSING, W., AND WRIGHT, A. W.: Changes in the connective tissue of the uterus and vagina of rat associated with advancing age. Science, 91: 577–578, 1940.
29. WOLFE, J. M., BURACK, E., LANSING, W., AND WRIGHT, A. W.: Effects of advancing age on the connective tissue of the uterus, cervix and vagina of the rat. Am. J. Anat., 70: 135–165, 1942.
30. ZONDEK, B., AND SKLOW, J.: Inactivation of estrone by liver after exclusion of the reticulo-endothelial system. Proc. Soc. Exper. Biol. and Med., 46: 276–278, 1941.

OBSERVATIONS ON THE OCCURRENCE OF VARICES OF THE ESOPHAGUS IN ROUTINE AUTOPSY MATERIAL*

TOBIAS WEINBERG, M.D.

From the Division of Pathology, Department of Laboratories, Sinai Hospital, Baltimore, Maryland

In the literature[1, 8] as well as in some textbooks,[7, 9–11] the statement is made that when varices occur in the upper part of the esophagus they are usually found in association with advanced age, obstruction of the superior vena cava or congestive heart failure. The statement is also made that varices in the lower part of the esophagus are due chiefly to cirrhosis of the liver or portal vein thrombosis.[1–3, 5, 8, 12]

Because a detailed analytic study of a series of consecutive autopsies could not be found and in order to determine the correctness of the statements made in the literature, a study was made of 1189 consecutive autopsies, exclusive of stillbirths (Table 1). All age groups were included because there are reports in the literature of esophageal varices in children.[6, 11] The table shows the number of autopsies by decades and the number in which varices were encountered in the corresponding decade. The presence of varices was determined by gross observation and confirmed by inspection of microscopic sections. No varices were found in children. The age of the youngest subject was 27, while that of the oldest was 100 years. Varices were most frequently encountered in the older age groups. There was a total of 95 cases in which varices were present, or 8.0 per cent of the autopsies studied. Varices were found twice as frequently in males (66) as in females (29).

The cases with varices were divided into groups, according to the associated major pathologic finding at necropsy. The results are shown in Table 2. In the miscellaneous group, there was one subject with chronic peptic ulcer of the stomach with bleeding, two with intracerebral hemorrhage and one each with the following associated major lesion: carcinoma of bladder, chronic peptic ulcer of duodenum with bleeding, adenocarcinoma of stomach with metastases to liver, adenocarcinoma of hepatic flexure of colon, follicular lymphoblastoma, carcinoma of adrenal cortex with metastases to liver, infectious polyneuronitis, massive pulmonary embolism following cholecystectomy, cor pulmonale, pyonephrosis, lymphosarcoma, adenocarcinoma of colon with metastases to liver, resection of adenocarcinoma of rectum with subsequent ileus, malignant Grawitz tumor of kidney with metastases to liver, lymphangiosarcoma of mesentery, and bronchiectasis and amyloidosis of kidneys. Except for those cases of carcinoma with extensive metastases to the liver there is little evidence for the production of a portal hypertension or even a mediastinal venous obstruction.

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, January 21, 1949.

Association of cirrhosis of liver with esophageal varices. It is commonly assumed that esophageal varices are present in most cases of Laennec's cirrhosis. In 13 of our 30 cases of Laennec's cirrhosis (43 per cent), in 4 of 6 cases of biliary cirrhosis and in 2 cases of marked cardiac pseudocirrhosis, varices were absent.

Table 3 lists the location of the varices in the esophagus in subjects with various diseases. It is evident that although the majority of patients with Laennec's cirrhosis show varices in the lower third of the esophagus the varices are not limited to the lower third, but may occur also in both the middle and upper thirds, even in the same case. On the other hand, when portal hypertension does not appear to be a factor the varices are not limited to the upper third of the esophagus, but are found to be distributed rather uniformly in all portions of the esophagus in the same case.

TABLE 1

INCIDENCE OF ESOPHAGEAL VARICES

	NO. OF AUTOPSIES BY DECADES	NO. WITH VARICES, BY DECADES	PER CENT
Up to 1 yr.	239	0	0.0
1–10 yrs.	28	0	0.0
11–20 yrs.	18	0	0.0
21–30 yrs.	44	1	2.3
31–40 yrs.	71	2	2.8
41–50 yrs.	151	8	5.3
51–60 yrs.	230	33	14.3
61–70 yrs.	245	30	12.2
71–80 yrs.	133	16	12.0
81–90 yrs.	27	4	14.8
91–100 yrs.	3	1	33.3
Total	1189*	95	8.0†

* 70 stillbirths were not included in this consecutive series of autopsies. † Average.

TABLE 2

GROUPING OF CASES WITH ESOPHAGEAL VARICES ACCORDING TO MAJOR PATHOLOGIC FINDINGS AT NECROPSY

	NO. OF CASES
Laennec's cirrhosis	17
Biliary cirrhosis	2
"Toxic" cirrhosis	1
Coronary heart disease	49
Rheumatic heart disease	2
Bronchogenic carcinoma with mediastinal mass	5
Miscellaneous diseases	19
Total	95

Actual number of varices. In 12 cases of Laennec's cirrhosis, the greatest actual number of varices encountered in the upper, middle and lower thirds of the esophagus was 2, 5 and 5 respectively; in 2 cases of biliary and in one case of toxic cirrhosis, the greatest actual number of varices was 9, 2 and 3; and in 35 other cases, the number of varices was not determined.

Occurrence of bleeding from esophageal varices. In 17 cases of Laennec's cirrhosis with varices, bleeding was present in 5 and absent in 12 cases. In 2 cases of biliary and one of "toxic" cirrhosis with esophageal varices, bleeding was absent in all 3 cases. In 75 other cases with varices, bleeding was present in only one case.

Although bleeding is commonly associated with Laennec's cirrhosis, its incidence in this series is not as great as one might expect. Occasionally, bleeding occurs in a patient in whom no reason for portal hypertension is apparent. One such patient in this series was a 49 year old white male who was suffering from chronic glomerulonephritis.

TABLE 3

LOCATION OF VARICES IN THE ESOPHAGUS IN SUBJECTS WITH VARIOUS DISEASES

CONDITION	NO. OF CASES	UPPER ⅓	MIDDLE ⅓	LOWER ⅓
Laennec's cirrhosis	15	3	8	14
Biliary cirrhosis	2	0	2	2
"Toxic" cirrhosis	1	1	1	0
Coronary heart disease	42	24	25	16
Rheumatic heart disease	1	0	1	1
Bronchogenic carcinoma with mediastinal mass	4	2	2	1
Miscellaneous diseases	18	11	6	6

In an analysis of the size of the varices, it was found that in Laennec's cirrhosis the size of the varices, on the average, was approximately three times as large as in noncirrhotic patients. It might be argued that the smaller varices tend to project less into the lumen and so the passage of food subjects them less to trauma and to resulting esophagitis and final erosion of the venous wall.

A consideration of the diseases in which esophageal varices were encountered in this series leads to conjecture as to the etiologic factors involved. One of the theories advanced[4, 7, 11] for the development of these varices is that foci of congenital weakness exist in the venous channels of the esophagus, the analogy being drawn to varices in the veins of the leg, the development of which is likewise popularly explained on the theory of congenital weakness. This theory is also used to explain the development of esophageal varices in older persons in whom there is altered hemodynamics, resulting in stasis, and so leading to malnourishment of the wall and finally to an ectasia or dilatation. The frequency with which varices were encountered in this series unassociated with either mediastinal or portal obstruction leads one to suspect that, although not demonstrated anatomically, there may be foci of congenital weakness within the venous wall

which become manifest upon the assumption of an additional burden by the wall in the form of increased tension, stasis or anoxemia.

SUMMARY

Although esophageal varices were found associated frequently with portal vein obstruction, they were also frequently encountered unassociated with such obstruction.

Categorical statements cannot be made as to localization of esophageal varices according to associated diseases inasmuch as varices are frequently present in all segments of the esophagus regardless of the underlying cause.

REFERENCES

1. Beutel, A.: Oesophagusvarizen. Acta radiol., **13**: 527–532, 1932.
2. Boyd, William: Pathology of Internal Diseases. Ed. 4. Philadelphia: Lea and Febiger, p. 322, 1944.
3. Christian, H. A.: Osler's Principles and Practice of Medicine. Ed. 14. New York: Appleton-Century Co., Inc., p. 38, 1942.
4. Friedman, E.: Esophageal varix; report of a case in a three and one-half year old child, not dependent on liver cirrhosis. J. Pediat., **4**: 641–647, 1934.
5. Gordin, A. E.: Esophageal and gastric varices. New Orleans M. and S. J., **84**: 865–868, 1932.
6. Hansson, C. J.: Varices of the oesophagus in children. Acta radiol., **25**: 507–513, 1944.
7. Henke, F., and Lubarsch, O.: Handbuch Der Speziellen Pathologischen Anatomie und Histologie. Vol. 4, part 1. Berlin: Julius Springer, pp. 110–112, 1926.
8. Hjelm, R.: Zwei Fälle von röntgendiagnostizierten Oesophagusvarizen. Acta. radiol., **12**: 146–150, 1931.
9. Karsner, H. T.: Human Pathology. Ed. 6. Philadelphia: J. B. Lippincott Company, p. 563, 1942.
10. Kaufmann, E.: Pathology for Students and Practitioners (translated by S. P. Reimann). Vol. 1. Philadelphia: The Blakiston Company, pp. 633–634, 1929.
11. Nochimowski, J.: Ueber tödliche Blutungen aus Oesophagusvarizen in Fällen ohne Lebercirrhose. Frankfurt. Ztschr. f. Path., **43**: 463–475, 1932.
12. Smith, L. W., and Gault, E.: Essentials of Pathology. Ed. 3. Philadelphia: The Blakiston Company, p. 425, 1948.

EPIDERMOID CYST OF THE SPLEEN

Report of a Case*

HERMAN J. LINN, M.D., and ELMER P. ELLIAS, M.D.

From the Departments of Pathology and Surgery of the Veterans Administration Hospital, Dearborn, and Wayne University College of Medicine, Detroit, Michigan

Cysts of the spleen lined by squamous epithelium are rarely encountered, and reports of only 12 such cases can be found in the literature. Hemorrhagic or serous splenic cysts lined by endothelium are, on the other hand, not uncommon. In this report of an epidermoid splenic cyst, we offer an explanation for their formation.

Previously reported cases are summarized in Table 1. The 5 cases mentioned by Custer[4] cannot be included because they are merely mentioned as occurring among 5000 routine autopsies. Lubarsch in discussing the case presented by Pohle[16] stated he had previously seen only one such case, probably that reported by Schneider.[18] The rarity of such cysts might lend doubt to the accuracy of Custer's report. It is noteworthy that most of these cysts occurred in patients between the ages of 14 and 22 years. A history of trauma was often obtained. Symptoms were usually of one or more years' duration and consisted of fullness in the left upper quadrant of the abdomen and sometimes of vague gastric complaints. Physical examination disclosed a mass in the left upper quadrant of the abdomen which moved with respiration, and which was almost always recognized as spleen. Treatment was splenectomy which was followed by little or no postoperative difficulty. Two patients [13, 17] were first treated with roentgen ray therapy without effect, one because of an erroneous diagnosis of myelogenous leukemia. The cyst was usually quite large before the patient sought medical advice and the spleen was removed. The following case is similar to those previously reported.

REPORT OF CASE

A 22 year old man was admitted to the Veterans Administration Hospital, Dearborn, Michigan, on June 12, 1947, with a history of recurrent attacks of right upper quadrant abdominal pain for three years. These attacks were accompanied by nausea, vomiting, chills, fever and sometimes jaundice. Twice he had been hospitalized for short periods. Two years previously his abdomen was injured in a football game, and he was hospitalized for observation for one night.

Physical examination upon admission to this hospital revealed a well nourished slightly icteric white man. The spleen was palpable three fingersbreadth below the left costal margin and descended to the pelvic brim on deep inspiration. There was slight tenderness

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

Received for publication, March 12, 1949.

in the right upper quadrant. All laboratory examinations gave normal results except for an icterus index of 9 units and a cephalin-flocculation test reported as one plus. Following roentgen demonstration of many calculi in the gallbladder, a cholecystectomy was performed on October 20, 1947. During the operation a large cystic spleen was found and 1600 cc. of dark greenish brown fluid containing black sediment was aspirated. Microscopically, blood pigment and cholesterol crystals were demonstrated in this fluid. During convalescence there was pneumonitis of the upper lobe of the right lung, but the patient soon became asymptomatic and was discharged October 31, 1947.

On June 12, 1948 he was readmitted because of recurrence of the mass in the left upper quadrant of the abdomen. On June 18 the abdomen was opened and 1000 cc. of clear straw-colored fluid was aspirated from a large cyst of the upper two thirds of the spleen and

TABLE 1

REPORTS OF EPIDERMOID SPLENIC CYSTS

AUTHOR	SEX	AGE	DURATION OF SYMPTOMS	SIZE (AND WEIGHT) OF SPLEEN	VOLUME (OR WEIGHT) OF CONTENTS
Bostick and Lucia[3]	M	20 yr.	5 yr.	20 x 10 x 6 cm. (1265 Gm.)	580 cc.
Dinand[5]	M	16 yr.	6 yr.	23 x 17 x 10 cm.	3000 Gm.
Gosset *et al.*[6]	F	16 yr.	11 mo.	22 x 18 x 14 cm.	2500 cc.
Harding[7]	F	22 yr.	7 yr.	9 cm.	—
Lereboullet *et al.*[12]	M	8 yr.	5 mo.	14 x 12 x 10 cm. (850 Gm.)	300 cc.
Montgomery *et al.*[13]					
Case 1	F	7 yr.	?	15 x 12 cm.	520 cc.
Case 2	M	6 mo.	?	4 x 2 x 1 cm.	—
Parker and Brown[15]	F	16 yr.	?	30 x 15 x 15 cm.	2000 cc.
Pohle[16]	F	19 yr.	9 yr.	31 x 22 x 14 cm. (5000 Gm.)	4000 cc.
Santy[17]	M	15 yr.	Many wks.	19 x 15 cm.	2700 cc.
Schneider[18]	M	14 yr.	1 yr.	Size of child's head (Over 3000 Gm.)	—
Shawan[19]	F	16 yr.	2½ yr.	22 x 17 x 15 cm.	1500 cc.

splenectomy was performed without difficulty. The postoperative course was uneventful and the patient was discharged on June 26, 1948.

On July 8, 1948 he was readmitted again because of pain in the left lateral lower thoracic and left lumbar regions. He had had a sore throat with associated chills and fever for one day, but when treated with penicillin he rapidly became asymptomatic and was discharged on July 15. On July 20, 1948 he was readmitted with severe pain in the left upper quadrant of the abdomen. A diagnosis of left subdiaphragmatic abscess was made and he was again treated with penicillin. On July 22, a left subdiaphragmatic abscess was incised and drained of 1500 cc. of pus. The abscess cavity closed rapidly and he was discharged on August 7, 1948. On September 9, 1948 the wound had entirely healed and he was gaining weight. When last seen in January 1949 he had had an attack of infectious hepatitis with jaundice and diarrhea since his discharge from this hospital, but had had no further complaints referable to the left upper quadrant of the abdomen.

PATHOLOGIC REPORT

The previously incised spleen weighed 472 Gm. and measured 18 x 11 x 5 cm. In its upper pole, completely surrounded by splenic parenchyma, was a cavity

measuring 8.5 cm. in diameter (Fig. 1). In places the surrounding splenic parenchyma was only 2 mm. in thickness. The lining of the cyst was shiny and irregularly corrugated, resembling the appearance of the endocardial surface of the left ventricle of the heart with its interlacing trabeculae carneae. The lining was in places roughened and varied from gray to yellow and purple with small areas of hemorrhage.

In microscopic sections, much of the cyst was found to be lined by stratified squamous epithelium with a distinct stratum granulosum and stratum corneum (Fig. 2). Often in the malpighian layer prickle cells with intercellular bridges were demonstrated (Fig. 3). In other areas the more superficial epithelial cells were vacuolated with intra-epithelial glands much like the glands of Littre of the posterior urethra (Fig. 4). No mucin could be demonstrated in these cells by appropriate staining.

Much of the epithelium, however, was missing from the lining of the cyst exposing a thick hyaline basement membrane. The remaining epithelium was loosely attached to this basement membrane, and no rete pegs or papillae were found. There were no hair follicles or sebaceous glands. The dense basement membrane was surrounded by a zone of loose connective tissue in which there were only few blood vessels. There was evidence of old hemorrhage both in this loose connective tissue, and in the surrounding splenic parenchyma.

About the large cyst, usually in trabeculae but also in the splenic pulp were about 12 smaller cysts measuring up to 3 mm. in diameter (Fig. 5). For the most part they were lined by a single layer of cells which had the appearance of cuboidal epithelium or mesothelium, but some small cysts had a lining from 3 to 5 cells in thickness. The small cysts contained pink-staining serum in which there were histiocytes with foamy cytoplasm which may have become detached from the mesothelial lining (Fig. 6). No transition from such mesothelial lining cells to squamous epithelial cells could be demonstrated.

THEORIES OF HISTOGENESIS

Kaijser[10] in a good review of epidermoid cysts of the spleen concludes that there is no satisfactory theory of their histogenesis, although many have been proposed. The commonest explanation is that epidermoid cysts arise from embryonic inclusions in the developing spleen. Lubarsch, as quoted by Pohle,[16] and Dinand[5] suggested the inclusion of germinal cells from the gonads, with one-sided development. This seems improbable, in that one would then expect to find occasional reports of true dermoid cysts of the spleen. Only 3 cases of doubtful or possible dermoid cysts of the spleen have been reported, but they are referred to by many authors as verified dermoid cysts. Kaijser[10] quotes Andral, who reported the first case, as follows: "The spleen may produce a very complicated cyst. I have seen one with a fibro-serous wall the interior of which contained fatty material, like tallow, in which were scattered some hairs." Kaijser adds, "A more complete description of this tumor cannot be found in the literature." Kumaris[11] described a dermoid cyst found in the lesser pelvis in a 40 year old woman. It was adherent to the mesentery and grossly contained hair.

At the lower pole of the cyst there was a thickened area which appeared to be spleen, the hilus of which served as the bed of the cyst. Kumaris himself says that it is impossible to be certain that the parenchyma of the spleen served as the origin of the dermoid. A third case of possible splenic dermoid cyst is referred to by Bostick and Lucia.[3]

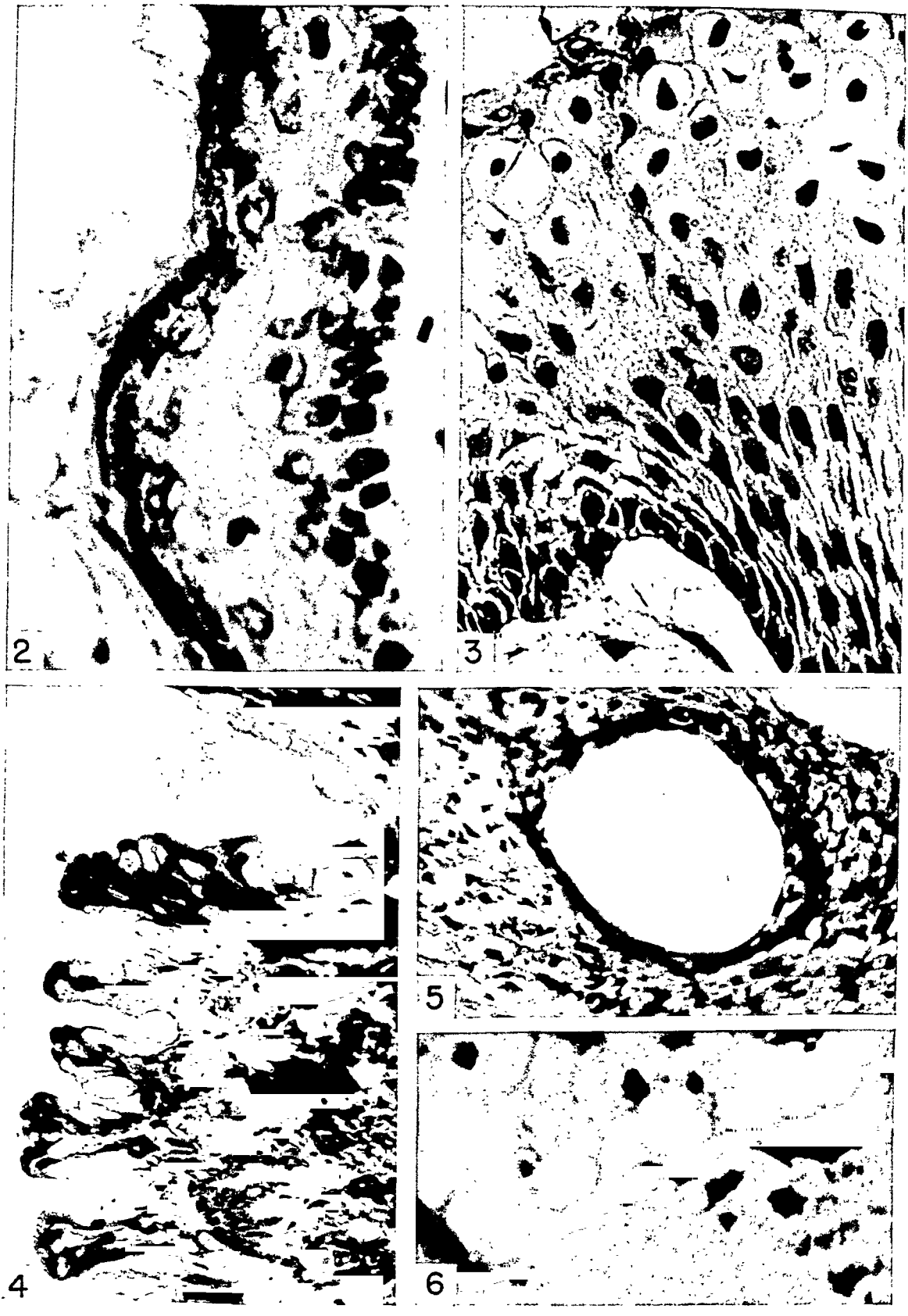
Inclusion of cells from the Wolffian duct which in the 10 mm. embryo is



FIG. 1. Cyst of the spleen seen from the hilar surface with the splenic notch in the upper right corner.

immediately adjacent to the spleen is suggested by Santy.[17] This theory also seems improbable in that more differentiated parts of the kidney or gonads have not been described in the spleen. Entodermal inclusions during the early development of the spleen in the dorsal mesogastrium are suggested by Schneider[18] and by Harding,[7] with subsequent squamous metaplasia. But why, then, have no mucus-producing cysts been described?

Pohle[16] suggested metaplasia of vascular endothelium or metaplasia of peritoneal inclusions. Many authors refer to Müller[14] who cited an instance of squamous metaplasia of the lining of an olecranon bursa. An illustration in



FIGS. 2-6

his article shows epithelium 6 to 8 cells in thickness with spinous processes between some of the cells, but no stratum granulosum or keratohyaline. However, in his case implantation of superficial skin into the underlying bursa certainly cannot be excluded.

Pohle's suggestion of metaplasia of peritoneal inclusions, on which he does not enlarge, appears to offer the most plausible explanation. Many authors[2, 8, 9, 20] have described the development of the spleen in the dorsal mesogastrium, both in man, and in the lower animals. During this development the cells lining the coelomic cavity over the rudimentary spleen (which cells we shall call mesothelium), retain their primitive character longer than the mesothelium of the peritoneal cavity in general. A basement membrane begins to form beneath these cells in the 7 to 15 mm. human embryo. Early, while the basement membrane is still incomplete, migration of the mesothelial cells into the underlying splenic anlage, or invagination of the mesothelial layer, can be seen and has been illustrated.[9, 20] Some of these cells, which may or may not be surrounded by the newly formed basement membrane, remain in the spleen, and probably give rise to epidermoid cysts of the spleen. The Wolffian duct arises by invagination of similar peritoneal mesothelium and gives rise to most of the excretory urinary passages. Squamous metaplasia of the pelvis of the kidney, the ureters or the urinary bladder is not uncommon. The Wolffian duct arises in the immediate vicinity of the primitive spleen, and it seems probable that similar mesothelial cells in the spleen might form cysts and undergo squamous metaplasia. It is doubtful that there is true inclusion of parts of the Wolffian duct, since no tubular structures have been described in the spleen. The intraepithelial goblet cells seen in the lining of the present cyst are, however, definitely suggestive of the glands of Littre of the posterior urethra.

The theory that epidermoid cysts of the spleen arise from mesothelial cells would explain why such cysts are sometimes multiple, though as in our case, usually only one is lined by squamous epithelium. Also it explains why hair and sebaceous glands are never found, which would sometimes occur if the cysts arose from germinal rests. In many cases, including ours, there is a history of previous trauma, and this factor cannot be excluded as contributing to the growth of the cysts.

SUMMARY

A large splenic cyst lined by keratinized stratified squamous epithelium is described, and the pertinent literature is cited. Reasons are advanced why this

FIG. 2. Stratified squamous epithelial lining of the cyst. Basal cells are arranged perpendicularly. The intermediate zone is edematous and there is a prominent stratum granulosum. The stratum corneum is becoming detached. × 750.

FIG. 3. Thicker squamous epithelium with a prominent prickle cell layer. The epithelium is partially detached from the basement membrane. × 500.

FIG. 4. Vacuolated superficial cells, morphologically similar to the glands of Littre. × 350.

FIG. 5. Smaller cyst lined by flattened cells of indeterminate nature. × 300.

FIG. 6. A small cyst lined by mesothelium. In it are histiocytes with foamy cytoplasm and pyknotic nuclei, probably desquamated mesothelial cells. × 725.

cyst is believed to have arisen by metaplasia from mesothelial cells lining the primitive coelomic cavity, which cells have been shown previously to migrate into the splenic anlage.

REFERENCES

1. ANDRAL, G.: Cited by Kaijser,[10] and Bostick and Lucia.[3]
2. BERGEL, A., AND GUT, H.: Zur Frühentwicklung der Milz beim Menschen. Ztschr. f. Anat. u. Entwcklngsgesch., **103**: 20–29, 1934.
3. BOSTICK, W. L., AND LUCIA, S. P.: Nonparasitic, noncancerous cystic tumors of the spleen. Arch. Path., **47**: 215–222, 1949.
4. CUSTER, R. P.: The Spleen. In Brennemann, J.: Practice of Pediatrics. Hagerstown, Md.: W. F. Prior Co., Vol. 3, Chapt. 20, p. 24, 1940.
5. DINAND, F.: Riesige epitheliale Solitärcyste der Milz. Arch. f. klin. Chir., **158**: 485–499, 1930.
6. GOSSET, A., BERTRAND, I., AND GOSSET, J.: Contribution à l'étude des kystes spléniques à revêtement épidermoïde. A propos d'un cas inédit. J. de chir., **54**: 289–303, 1939.
7. HARDING, H. E.: Large inclusion cyst in spleen. J. Path. and Bact., **36**: 485, 1933.
8. HARTMANN, A.: Die Entwicklung der Milz. Handbuch der mikroskopischen Anatomie des Menschen. Vol. VI, part 1, pp. 529–540, 1930.
9. HOLYOKE, E. A.: Rôle of primitive mesothelium in development of mammalian spleen. Anat. Rec., **65**: 333–349, 1936.
10. KAIJSER, R.: Über zystische Milztumoren epithelialen Ursprungs. Upsala läkaref. förh., **45**: 261–284, 1939.
11. KUMARIS, J.: Milzdermoid und Wandermilz. Arch f. klin. Chir., **106**: 699–705, 1915.
12. LEREBOULLET, P., GREGOIRE, R., BERNARD, J., AND IBARRA, M. R.: Les kystes épidermoïdes de la rate. Sang, **13**: 853–869, 1939.
13. MONTGOMERY, A. H., MCENERY, E. T., AND FRANK, A. A.: Epidermoid cysts of spleen. Ann. Surg., **108**: 877–884, 1938.
14. MÜLLER, H.: Die histologische Übereinstimmung zwischen Epithelregeneration und Krebsbildung. Ztschr. f. Krebsforsch., **28**: 383–417, 1929.
15. PARKER, E. F., AND BROWN, A. G.: Epidermoid cyst of the spleen. Surgery, **24**: 708–713, 1948.
16. POHLE, W.: Über Milzcysten. Mit besonderer Berücksichtigung eines Falles von grosser Milzcyste bei Paratyphuserkrankung. Deutsch. Ztschr. f. Chir., **221**: 211–222, 1929.
17. SANTY, P.: Splénectomie pour un kyste vrai de la rate chez un enfant. Lyon chir., **27**: 101–104, 1930.
18. SCHNEIDER, P.: Eine splenomegalische Epidermoidzyste. Verhandl. d. deutsch. path. Gesellsch., **24**: 280–281, 1929.
19. SHAWAN, H. K.: Epidermoid cysts of spleen. Arch. Surg., **27**: 63–74, 1933.
20. THIEL, G. A., AND DOWNEY, H.: Development of mammalian spleen, with special reference to its hematopoietic activity. Am. J. Anat., **28**: 279–339, 1921.

SIMULATED HOMICIDE

Report of a Case of Obstructive Laryngeal Papilloma in a Child*

JOSEPH W. SPELMAN, M.D.†

From the Department of Legal Medicine, Harvard University Medical School, Boston, Massachusetts

Circumstantial evidence combined with incomplete or incompetent medical investigation frequently predisposes to the erroneous suspicion that death has resulted from homicide. If the autopsy in the case herewith reported had been terminated with the demonstration of recent and severe cranio-cerebral trauma, death would probably have been attributed to homicide. The finding during the subsequent course of the autopsy that the larynx had been completely and acutely obstructed by an edematous papilloma established that death had resulted from natural causes and provided a reasonable explanation of the manner in which the trauma was sustained.

REPORT OF CASE

History

Early one morning, the police were notified that an apparently intoxicated, Negro man accompanied by two women had been seen carrying through the streets, a carelessly wrapped parcel which appeared to contain the body of a dead child. A passerby reported overhearing the trio discussing the best means of disposing of the corpse. On arrival of the police, the man stated that the body was that of his 16 month old son. The body was immediately taken to a mortuary by the police and the medical examiner notified.‡

It was learned that the mother of the dead child was, and had been for several days, a patient in a neighboring hospital. Statements of the father and the women accompanying him concerning the child's death were conflicting. The father stated that he had put the boy to bed on a sofa in the family living-room at about 10:00 o'clock the night before. He had then gone to the apartment of one of the women, became intoxicated, and returned home at 2:30 A.M. to find his son dead on the floor near the sofa. After a conference between him, his companion of the night and another woman, the three of them wrapped the body in a blanket and were proceeding along the street in an aimless fashion when detected at approximately 3:00 A.M.

* Received for publication, February 14, 1949.

† Present address: Office of State Pathologist, Department of Public Health, Burlington, Vermont, and College of Medicine, University of Vermont, Burlington, Vermont.

‡ The Department of Legal Medicine was invited to assist in the investigation of this case by Andrew D. Guthrie, M.D., Medical Examiner of Medford, Massachusetts.

Postmortem Examination

The examination was performed late that same afternoon. The body was dressed in urine-soaked, daytime clothing. The shoes had been placed on the wrong feet and were laced but not tied. The body was undernourished and underdeveloped, measured 63 cm. in length and was estimated to weigh not more than 7 Kg. There was no external evidence of recent trauma.

Incision of the body disclosed marginal pulmonary emphysema and occasional subpleural petechiae. The thoracic and abdominal viscera were not otherwise remarkable. Upon reflecting the scalp, a large transverse linear fracture of the right parietal bone was immediately apparent. The fracture measured 6 cm.



FIG. 1. Larynx opened posteriorly showing obstructing papilloma. $\frac{2}{3}$ normal size.

in length, and was covered by a fresh hematoma, 2 mm. in thickness, which elevated the pericranium over an area measuring 6 cm. by 4 cm. A scanty epidural hemorrhagic extravasation surrounded the fracture. There were a few superficial petechiae throughout the cerebral cortex. The intracranial contents were not otherwise notable. If the autopsy had been concluded at this point, there would have been a strong presumption that the child had died as a result of a fractured skull with associated cerebral damage. Examination of the organs of the neck, however, disclosed the larynx to be completely obstructed by multiple papillomatous tumors. The soft, friable, polypoid masses obliterated the vocal cords and extended from the ventricular ligaments to the inferior margin of the cricoid cartilage (Fig. 1). Firmly attached to the entire circumference, the neo-

FIG. 2. Low power photomicrograph revealing papillary tumor of laryngeal mucosa. H. and E. × 15.

FIG. 3. Higher power of tumor showing nature of epithelium and inflammatory reaction. H. and E. × 42.





FIGS. 2-3

plasm had displaced the submucosa and had penetrated to, but not into, the laryngeal cartilages.

Microscopic observations. The tumor of the *larynx* was composed of thick folds of well-differentiated squamous epithelium disposed in the form of branching papillae supported by an edematous stroma. In places the submucosa was displaced and the tumor reached the perichondrium. It was everywhere confined within a well-defined basal membrane. There was moderate hyperemia, edema and round cell infiltration of both the tumor and the adjacent mucosa. Inclusion bodies were not seen (Figs. 2 and 3). In the *brain*, occasional hemorrhagic extravasations into the Virchow-Robin spaces were present. Small focal hemorrhages without exudation were noted in the *lungs*. The microscopic appearances of the other organs were not unusual.

Chemical examination. Blood taken from the right and left chambers of the heart contained, respectively, 476 mg. and 310 mg. of glucose per 100 ml.; a finding which was regarded as consistent with, although not diagnostic of, death by obstructive asphyxia.[4]

Additional history. It was learned subsequently that the decedent had been examined in a children's clinic at the age of 10 months because of weight loss and cough. At the time, hoarseness, muffled voice, cough and weight loss had been noted and the diagnoses of malnutrition, rickets and upper respiratory infection were made. When seen again at 13 months of age, his weight had increased from 4.3 Kg. to 5.1 Kg., and the infection had disappeared. Observations concerning his muffled voice and hoarseness were not recorded at that time. The larynx had never been directly examined. He was not seen again at the clinic.

COMMENT

The circumstances of an intoxicated, philandering father, carrying the dead and partially concealed body of a neglected child through the streets aroused suspicion. The finding at autopsy of a fractured skull enhanced this suspicion and might well have led to a charge of homicide. Finding the larynx completely obstructed by tumor later in the examination, however, dispelled all such inferences. The fracture could now be explained as having been sustained during the agonal period either from a fall from the sofa, or from trauma received during a terminal asphyxial convulsion. This case convincingly illustrates the necessity for performing complete and thorough medicolegal autopsies.

According to Jackson and Jackson,[5] the occurrence of laryngeal papillomas in early childhood is by no means rare. Farber[2] reports that in a series of 15,293 consecutive surgical specimens studied between 1916 and 1947 at the Children's Hospital in Boston, there were 92 laryngeal papillomata, an apparent incidence of approximately 1 in 160. Since these figures include all specimens from some patients subjected to multiple biopsies the actual incidence in surgical cases is somewhat lower.

Papillomas of the larynx in children are characteristically benign, often recur, and usually disappear at puberty.[5, 3] Although benign, they frequently cause death by asphyxia. Crowe and Breitstein[1] state that the mortality rate from

papillomas of the larynx in children under 5 years of age is at least equal to that of carcinoma of the larynx in adults. Bruns, cited by Summers[6], recorded 28 deaths by suffocation in a series of 48 untreated cases of laryngeal papillomas. Recently, Ferguson and Scott[3] reported 3 acute asphyxial deaths in a series of 15 cases.

It should be noted that the tumor in the present case was accompanied by considerably more than the usual mild inflammatory reaction seen in such lesions. It appeared that the edema, hyperemia and exudation incident to an upper respiratory infection, superimposed on pre-existing, partial, laryngeal obstruction, resulted in this sudden occlusion.

SUMMARY

A study is presented of a case of sudden death in childhood which was followed by circumstances that provoked suspicion of murder. The discovery of a fractured skull and associated cerebral damage would have enhanced the suspicion of death by homicide had not the obstructing laryngeal papilloma been demonstrated.

The constant threat of asphyxia from this common tumor is discussed.

REFERENCES

1. Crowe, S. J., and Breitstein, M. L.: Papilloma of the larynx in children. Arch. Surg., **4**: 275–299, 1922.
2. Farber, S.: Personal communication to the author.
3. Ferguson, C. F., and Scott, H. W., Jr.: Papillomatosis of the larynx in childhood. New England J. Med., **230**: 477–482, 1944.
4. Hill, E.V.: Significance of dextrose and nondextrose reducing substances in postmortem blood. Arch. Path., **32**: 452–473, 1941.
5. Jackson, C., and Jackson, C. L.: Diseases and Injuries of the Larynx. Ed. 2. New York: The MacMillan Company, 633 pp., 1942.
6. Summers, J. E., Jr.: The treatment of papilloma of the larynx in children. New York M. J., **78**: 357–359, 1903.

GAUCHER'S DISEASE IN A NEGRO

Report of Case*

ROGER M. CHOISSER, M.D., and ROBERT R. MONTGOMERY, M.D.

From the Pathology Department, George Washington University School of Medicine, Washington, D. C.

This report is concerned with the occurrence of Gaucher's in a Negro. Only two other reports of the disease in Negro patients could be found in the literature.[1, 3]

CLINICAL DATA

First Admission

Present illness. A 21 year old Negro woman was admitted to Freedmen's Hospital, Washington, D. C., on August 19, 1946, with a chief complaint of prolonged menstrual bleeding, present for about five weeks. During the last few days prior to admission, bleeding was so profuse as to require twelve napkins daily. The only associated symptoms were progressive weakness and dizziness with some frequency of urination, and transitory edema of the ankles two days before admission. Previously there had never been any menstrual abnormalities. Menses began at 12 years, occurred at intervals of 30 days and lasted five to six days. She had never been pregnant. She had noted a painless swelling in the left side of the abdomen for several months. Appetite had been good and there was no nausea, vomiting or change in bowel habits. There was no change in weight.

Past history. She had the usual childhood diseases with no complications. In 1944, she had a pleural effusion. X-ray examination of the chest after recovery was reported to her as negative.

Family history. Her father died of hypertension; mother was living and well at age 62; one brother has tuberculosis; two sisters and five other brothers are living and well.

Physical examination. The patient was fairly well developed and well nourished but extremely pale. Temperature was 100 F., pulse rate 124, respiratory rate 36, and blood pressure 140/60. The skin was pale brown and presented no lesions. The sclerae and conjunctivae were clear. The mucous membranes of the nose and throat were pale. Examination of the chest was negative. The abdomen was rounded and not rigid. In the left upper quadrant was a firm, nontender mass which moved with respiration and extended to the umbilicus. A notch was palpated on its border. In the right upper quadrant was another palpable mass 4 cm. below the costal margin which also moved with respiration and which was moderately tender. There was moderate vaginal bleeding. Pelvic examination was negative. The extremities presented no abnormalities.

Laboratory data. The red blood count was 1,300,000; hemoglobin 20 per cent; leukocyte count 8400; polymorphonuclear leukocytes 56, band forms 3, myelocytes 3, myeloblasts 3, eosinophils 1, lymphocytes 33, monocytes 1. Bleeding and clotting times, fragility test, and test for sickling phenomenon were all normal. Blood phosphorus 2.4 mg. per 100 ml., calcium 8 mg., bilirubin 0.25 mg., nonprotein nitrogen 24 mg., sugar 86 mg.; icterus index 9 units, total cholesterol 91 mg., cholesterol ester 59 per cent of total; total cholesterol (second test) 110 mg., alkaline phosphatase 3.3 Bodansky units, total protein 7.88 Gm.; cephalin flocculation 4 plus in 24 hours; prothrombin time 80 per cent of normal. Urinaly-

* Read before the Washington Society of Pathologists, Washington, D. C., December 4, 1948.

Received for publication, January 10, 1949.

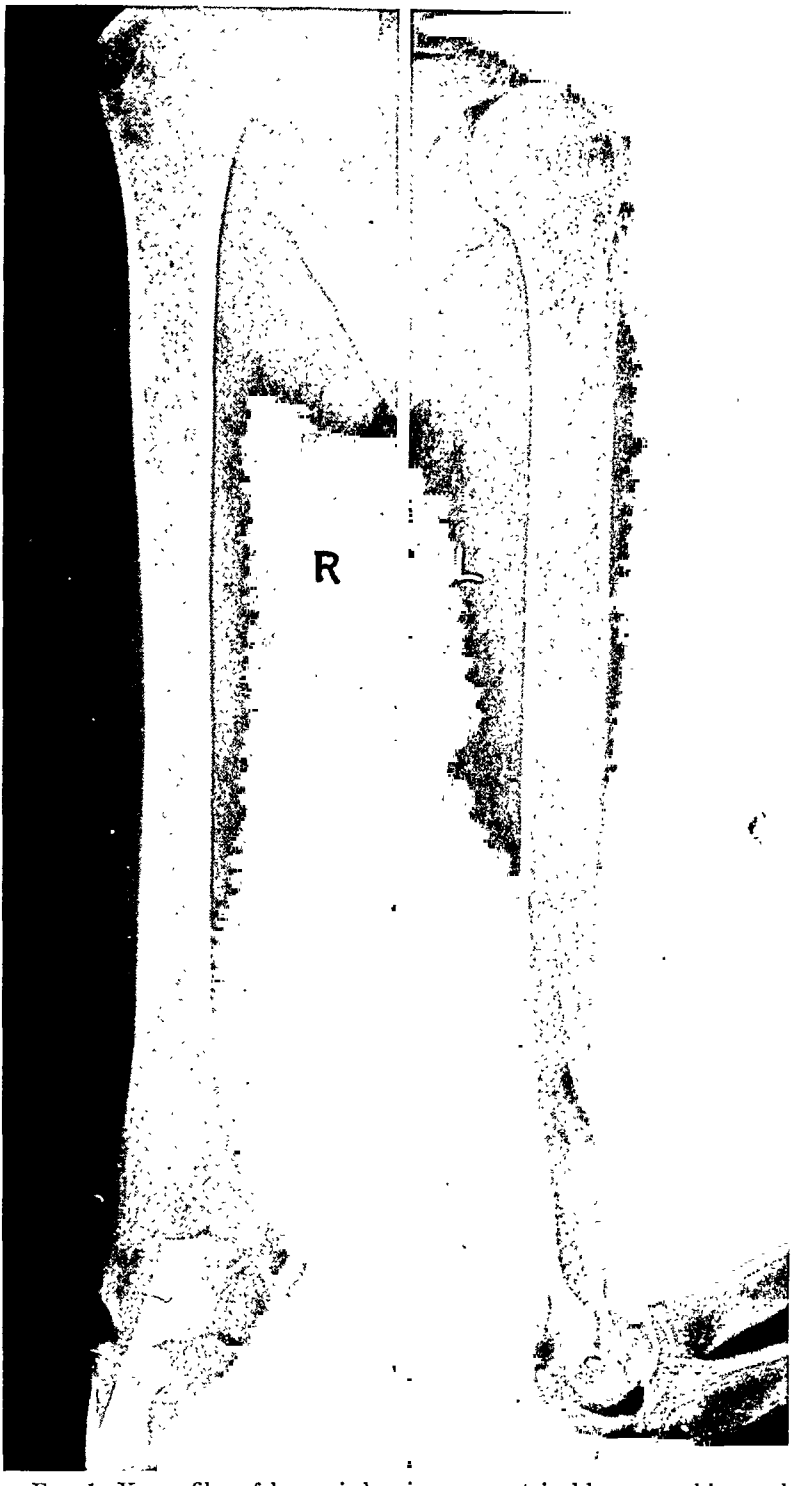


FIG. 1. X-ray film of humeri showing symmetrical honeycombing and widening of the medulla.

sis was negative except for a trace of albumin and many red blood cells. Smears of gastric washings revealed no acid-fast bacteria.

Electrocardiogram. P-R interval 0.14 seconds, QRS 0.06 seconds, left axis deviation, rate 115, moderate slurring of QRS in leads 1, 2 and 3, CF_1 and CF_2, QRS abnormally small in CF_2 and of "W" type, ST_1 and ST_2 mildly depressed, ST_3 elevated, T_3 tall and upright, T CF_1 inverted, T CF_2 and T CF_4 upright. Interpretation: "Findings strongly suggest myocardial damage."

*X-ray films of chest:** "Both leaves of the diaphragm are high and there is a mild congestion throughout both lungs. Heart has a mitral configuration and is enlarged, measuring 14 cm. in its transverse diameter with a transthoracic diameter of 26 cm. Impression: congestion, both lung fields; mild cardiac enlargement. Examination of both upper extremities and adjacent portions of the shoulders shows an increase in the width of the medullary canals. The humeri appear honeycombed and present an unusual pattern of decalcification (Fig. 1). Changes are fairly symmetrical and are consistent with some of the unusual forms of anemia. Skull films show no abnormalities."

Course in hospital. The temperature was irregular throughout her hospital stay, being normal for a few days, then elevated between 101 and 103 F. for one or two days. The vaginal bleeding stopped shortly after admission, but bleeding per rectum was noted for a few days during the second week. The anemia gradually improved after administration of a total of 1,500 ml. of whole blood and 500 ml. of plasma; on October 7, the red blood cell count was 3,230,000, and she felt better. She was given 156 r of x-ray over the spleen with no effect. A sternal puncture was unsatisfactory, and was not repeated. The diagnosis was Banti's syndrome, and splenectomy was recommended, but the patient refused. She was released from the hospital on October 7, 1946.

Second Admission

Present illness. She was admitted to Gallinger Municipal Hospital, Washington, D. C., on May 15, 1948. During the previous 19 months, the menses were fairly regular but quite profuse. She exhibited other bleeding tendencies such as severe epistaxes, melena, and severe bruising on slight trauma. She became progressively weaker. The weakness was now so severe that she was unable to be up and about for more than a few hours at a time. She fainted two days before admission. For several weeks she had anorexia, ankle edema, palpitation, dyspnea and mild orthopnea.

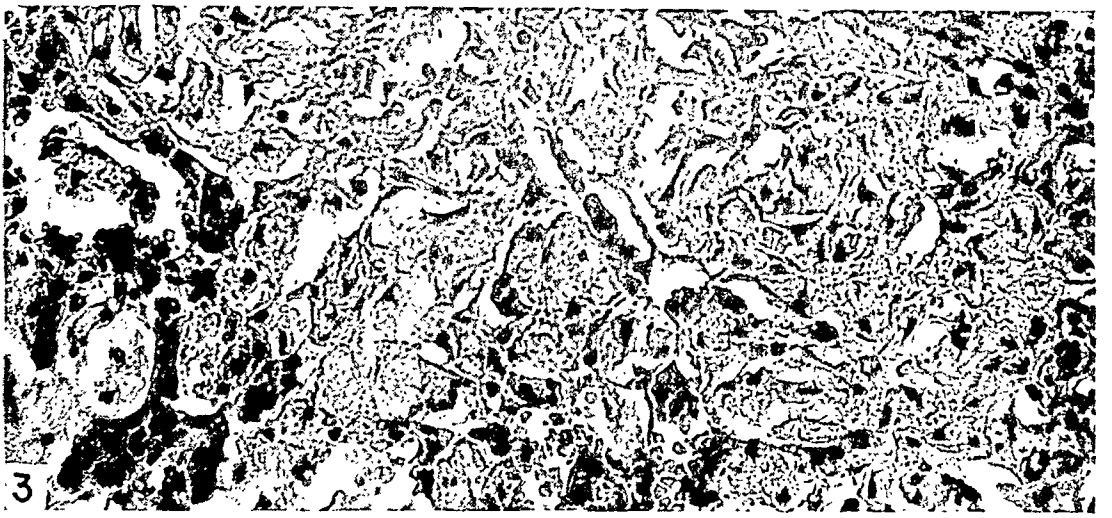
Physical examination. The patient was extremely ill, pale, weak, emaciated, and preferred to sit up in bed. The temperature was 100 F., pulse rate 100, respiratory rate 24, blood pressure 95/45. There were a few ecchymoses over the tibias. All mucous membranes were pale. No superficial lymph nodes were palpable. Examination of the lungs revealed dullness to percussion; many moist rales were audible over the lower lobes. The heart was not enlarged, the rhythm was regular, and the rate was 100 per minute. A coarse, systolic murmur was heard over the apex and not transmitted. In the abdomen a large, firm mass was felt on the left side, which extended from the costal margin to the iliac crest and across the midline at the umbilicus. A notch was felt on its edge. It was nontender and seemed to move on respiration. In the right upper quadrant dullness on percussion was noted 4 to 5 cm. below the costal margin, but no definite mass was outlined. The extremities were severely edematous.

Laboratory data. The urine contained a trace of albumin. The red blood cell count was 1,230,000, hemoglobin 3.5 Gm.; leukocyte count 5600; polymorphonuclear leukocytes 70, band forms 8, juvenile forms 5, basophils 1, lymphocytes 13, monocytes 3. Venous pressure and circulation time were normal: bleeding time 6 minutes.

Course in hospital. On admittance, she was almost exsanguinated and *in extremis*. She was given a transfusion of 500 ml. of whole blood, but died on the first day of hospitalization.

* Courtesy of Dr. C. H. Kelley, Freedmen's Hospital, Washington, D. C.





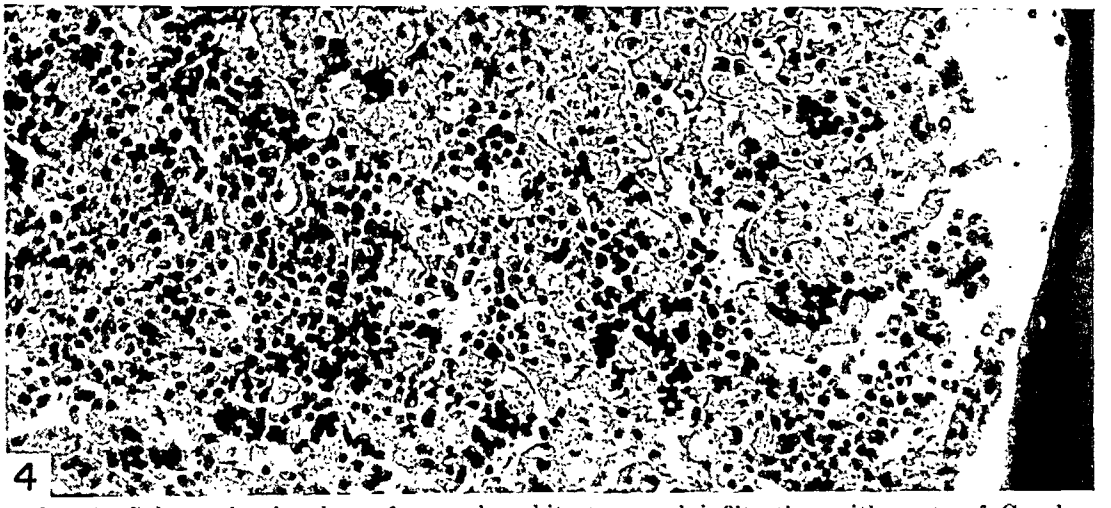


FIG. 2. Spleen, showing loss of normal architecture and infiltration with nests of Gaucher cells. × 100.

FIG. 3. Liver, showing loss of lobular architecture with infiltration by the Gaucher cells. Only thin strands of normal parenchyma remain. × 100.

FIG. 4. Vertebral marrow showing infiltration of the Gaucher cells. × 100.

AUTOPSY FINDINGS

Gross. The body was that of a somewhat underdeveloped, emaciated, pale, Negro female. The abdomen was rounded and firm. There was severe pretibial edema, and many petechiae were seen on the shins and under the nails. The mucous membranes of the eyes, nose and throat were all very pale, and a few splinter hemorrhages were present on the gums. The peritoneal cavity contained about 500 ml. of straw-colored, fibrinous fluid. There were many fibrinous adhesions throughout the abdomen dividing it into a loculated cavity. The spleen almost filled the left side of the abdomen, extending 17 cm. below the costal margin. It was very firm, pale, pinkish gray, weighed 3450 Gm., and cut with markedly increased resistance, revealing a dry, homogeneous, dusky pink cut surface. It contained many white, wedge-shaped areas with their bases at the capsule and many scattered yellowish nodules, varying in size from a pinhead to a barley grain and having soft, cheesy centers. The trabeculae and Malpighian bodies were not visualized. The liver was also enlarged, its edge extending 7 cm. below the costal margin. It weighed 2950 Gm., was dusky tan in color, moderately soft, and presented blunted edges. It cut with little increase in resistance and its cut surface was mottled yellow and tan and was moderately congested. The mesenteric lymph nodes and those in the aortic chain were enlarged up to 3 cm. in their long axis. They were rubbery and pale yellowish white on section. The other abdominal organs presented no abnormalities. Both pleural surfaces presented many fibrinous adhesions, and the right pleural cavity contained approximately 50 ml. of serofibrinous fluid. The lungs were markedly edematous. The heart was of usual size and quite flabby. The pelvic organs were densely matted with adhesions. Both ovaries consisted of thick, hollow shells with green, shaggy linings and contained chocolate colored, dirty, foul-smelling material.

Microscopic examination of the *spleen* revealed complete loss of the normal architecture. Almost the entire spleen was replaced by an infiltration of foreign cells arranged in nests resembling solid acini (Fig. 2). Each such group contained 4 to 25 cells and was well supplied with blood by a fine vascular stroma coursing between the cells. These were typical Gaucher cells, large, eosinophilic and presenting fine fibrils running through the cytoplasm to form an anastomosing network. The nuclei were fairly small in comparison to the size of the cells and were eccentric. Many cells were multinuclear, containing as many as 6 nuclei. No mitotic figures were seen. Thin strands of red and white pulp were seen between some of the nests. In one section was a large anemic infarct in which mere ghosts of the invading cells could be seen. In several sections were small tubercles with caseation necrosis surrounded by a few epithelioid cells and Langhans type of giant cells. Sections of the *liver* revealed a complete loss of the lobular architecture. Cells identical with those in the spleen were widely distributed through the liver in nests, sheets and strands. They did not follow the path of periportal fibrous tissue, but invaded the lobules so diffusely that no lobular outline was discernible, the parenchymal cells of the liver being compressed and atrophic in many areas (Fig. 3). Sections of the *lymph nodes* and *bone marrow* also showed extensive infiltration with the Gaucher cells (Fig. 4). The lymph nodes, in addition, presented many typical tubercles. The serosa of the *bowel* was studded with tubercles, and the *ovaries* were almost completely replaced by tuberculosis. No other areas of Gaucher cell infiltration could be found. Efforts to identify kerasin in the tissues by the method of Kahn and Kantrowitz[2] were unsuccessful.

DISCUSSION

This patient had classical Gaucher's disease, proved at autopsy. Her entire clinical picture including the laboratory and x-ray findings supported the diagnosis. The progress of the disease was slow and death was caused by an intercurrent disease, namely, tuberculous oophoritis and peritonitis. Although Gaucher's disease was suggested by one examiner during her first hospitalization,

it was ruled out on the basis of race. The x-ray findings were typical, but the roentgenologist also ruled out this diagnosis because of race. If the sternal puncture had been technically satisfactory, the diagnosis would no doubt have been made. Unfortunately, the patient was released from the hospital before another puncture could be made.

In reviewing the family history with the mother, it was denied that any matings had taken place with members of the Caucasian race. Both parents of the patient were, as far as could be determined, Negro. The grandparents were also Negro. Examinations could not be made of the patient's siblings, as they had moved to several different cities. No other members of the patient's family, however, were known to have enlargement of the spleen, swelling of the abdomen, or other finding suggestive of this disease.

SUMMARY

A case of Gaucher's disease is reported in a full-blooded Negro woman of 21.

REFERENCES

1. HOFFMAN, S. J., AND MAKLER, M. I.: Gaucher's disease; review of literature and report of case diagnosed from section of inguinal lymph gland. Am. J. Dis. Child., **38:** 775–793, 1929.
2. KAHN, J., AND KANTROWITZ, A. R.: Gaucher's disease; histochemical demonstration of kerasin in tissue. Am. J. Path., **22:** 653, 1946.
3. POTTER, E. B., AND MCRAE, C. C.: Gaucher's disease; report of 2 cases with remission in one following administration of liver extract. Am. J. M. Sc., **185:** 92–96, 1933.

CLINICOPATHOLOGIC CONFERENCE*

BÉLA HALPERT, M.D., AND S. N. STONE, M.D.

From the Department of Pathology and the Department of Surgery, the University of Oklahoma School of Medicine and the University of Oklahoma Hospitals, Oklahoma City, Oklahoma

Dr. Halpert. "An exploratory laparotomy is indicated whenever there is a condition in the abdomen which cannot be accurately determined by other clinical means or by laboratory methods. Such an exploration may reveal a diseased organ which can be removed and the patient thereby cured, or a disturbed function which may be corrected by an operative procedure. On the other hand, if the condition is beyond operative correction, corroborating evidence may be obtained by biopsy of a specimen of tissue. The complexity of the problem is well illustrated by the case to be discussed. Dr. Stone will present and analyze the clinical data."

CLINICAL DATA

The patient, F. D., a white male, aged 48, was admitted February 8 and died March 1, 1947.

Chief complaint. Indigestion, abdominal pain, vomiting.

Present illness. The patient stated that he began to have frequent attacks of indigestion three years ago, usually following ingestion of food, with epigastric fullness, slight pain and belching. These symptoms continued, becoming more frequent and severe. In October, 1946, the sensation of epigastric fullness persisted for four to five hours after meals and was relieved only by vomiting. The vomitus consisted of recently ingested food and did not contain any bright or dark blood. In the five months preceding admission the patient lost 47 pounds, had some pain in the left lumbar region, and nocturia four or five times. In January, 1947, he noticed a firm, tender mass in the upper part of the abdomen. His physician prescribed some powders which gave slight relief. After x-ray films were made of the stomach, he was advised to come to the University of Oklahoma Hospitals. At the time of admission he was able to ingest only liquid and soft foods.

Past and family history. When 11 years old, the patient had rheumatism with swelling of both knees and ankles, remained in bed one week and thereafter had no residual disease. He had gonorrhea in 1922 but denied ever having had syphilis. His mother died of cancer of the stomach at the age of 54.

Physical examination. At the time of admission, the patient was well developed, poorly nourished, and appeared chronically ill. No cervical nodes were palpated. No abnormalities of the chest were noted on auscultation and percussion. The heart tones were faint, apical systolic and diastolic murmurs and an aortic systolic murmur were heard, the pulse was regular and the rate ranged from 80 to 100. The blood pressure was 110/70. There was no abdominal

* Received for publication, February 5, 1949.

distension or rigidity. A visible and palpable mass in the left epigastrium was firm, tender, movable and transmitted the aortic pulsations. The edge of the liver extended 5 cm. below the costal margin in the right midclavicular line. The external genitalia were negative. Rectal examination revealed tenderness and protruding hemorrhoidal nodules; a "shelf" was palpable above the prostate along the anterior wall.

Laboratory data. Urinalysis: occasional erythrocytes and many leukocytes. Examinations of blood: erythrocytes, 3,890,000 per cu. mm.; hemoglobin 8 Gm. per 100 ml.; leukocytes, 10,850 per cu. mm. (No differential counts recorded); Mazzini test, negative; nonprotein nitrogen, 52.6 mg.; plasma proteins 6.25 Gm. per 100 ml., albumin/globulin ratio, 2.6:1; prothrombin time, 103 per cent of normal. Two gastric analyses: traces of blood, 35 and 80 degrees of total acid.

A photofluoroscopic examination of the chest on February 6 revealed elevation of the left leaf of the diaphragm. Gastrointestinal series disclosed an extrinsic or intrinsic tumor involving the cardia of the stomach. A urogram revealed distorted calices in both kidneys, diverticula of the urinary bladder and some calcifications in the right upper quadrant of the abdomen. On February 12, an electrocardiogram revealed suggestive evidence of low-grade left ventricular preponderance.

Clinical course. He was given general dietary and supportive treatment and whole blood transfusions. On February 23, pitting edema of the feet and ascites were noted. On February 24, laparotomy disclosed a mass involving the cardia of the stomach, the spleen inseparable from the greater curvature, and numerous enlarged lymph nodes and peritoneal implants. One of the nodes in the gastrocolic ligament and a section of tissue from the liver were taken for microscopic study. On February 28, numerous musical rales were noted in the right lung, and there were diminished to absent breath sounds with dullness to percussion over the left base. The temperature was 99 F. His condition became worse, and he died March 1, 1947.

CLINICAL DISCUSSION

Dr. Stone. "The patient was near the age group in which malignant disease is common. Vomiting was apparently frequent and suggested some interference with the motility of the stomach. It usually occurred about four hours after ingestion of food. This means that the disturbance caused gastric retention for about four hours, necessitating emptying of the stomach by vomiting, thereby relieving discomfort and pain. There was no history of vomiting of blood. This is significant as to the type of lesion. About two months before admission, a mass was noted in the upper portion of the abdomen, in the region of the stomach. This, together with the marked loss of weight, suggests the presence of a cancer.

"The nocturia and left lumbar pain I am at a loss to explain, except as due to some secondary condition. The family and past histories were not remarkable except for the mention of rheumatic fever. According to the physical examination, he was poorly nourished and appeared chronically ill. The systolic and diastolic murmurs heard at the apex and over the aortic area may have been the

result of the malnutrition and anemia or may have been connected with the rheumatic fever.

"The visible, palpable, round, tender, movable mass in the region of the stomach suggests a growth in the stomach. Malignant growths in the upper abdomen, other than those of the stomach, are usually not movable. Transmission of aortic pulsations is to be expected of a mass in this situation in a thin person. Palpation of a 'shelf' in the anterior wall of the rectum indicates that there is neoplastic involvement of the pelvic peritoneum along its reflection.

"The patient had an anemia with the red blood cell count just below normal. The hemoglobin value was 8 Gm., the blood nonprotein nitrogen was elevated and the plasma proteins were near normal level. In an emaciated patient with a debilitating illness or malignant tumor, one may be misled by the plasma protein values. There is a decreased blood volume in such patients so that the total circulating plasma proteins are actually less than the values would indicate. Although the plasma protein in our patient was near normal level, he had edema before and after operation, apparently due to hypoproteinemia. The gastric content showed traces of blood. This might or might not have been due to trauma but usually indicates an intrinsic lesion of the stomach. The total gastric acidity was normal. Achlorhydria occurs in the majority of patients with gastric carcinoma, but nearly normal values may be observed. Photofluoroscopic studies of the chest disclosed that the left leaf of the diaphragm was elevated. This suggests that the process had extended outside the stomach. The roentgenologist discovered an extrinsic or intrinsic tumor involving the cardia of the stomach leading me to suspect that the lesion was advanced. If the roentgenologist was not sure whether the lesion was intrinsic or extrinsic it would indicate that the lesion was not an ordinary carcinoma of the stomach. A carcinoma of the stomach that is sufficiently advanced to produce a palpable mass, should have a characteristic roentgenologic appearance. Since its appearance was not characteristic, we may conclude that this neoplasm was of an unusual type. The urograms revealed distorted calices in both kidneys. One might explain the distortion as caused by neoplastic tissue replacing the renal parenchyma and changing the normal contour of the calices. The presence of ascites together with a mass in the abdomen is certainly indicative of malignant disease. Since the findings are not typical of carcinoma, we must consider some of the other malignant tumors of the stomach such as the sarcomas. Of the sarcomas, lymphosarcoma is the most common and could have produced this picture. Such a lesion at the cardia of the stomach could, by direct extension, involve adjacent organs such as the spleen, yet not be obvious upon roent-

FIG. 1. Lymphosarcoma of the stomach (N-13-47) in a white male aged 48. The entire stomach, except 4 cm. of the pyloric end, was involved causing thickening of the wall up to 2.5 cm. along the greater curvature. The pattern of the mucosa as well as that of the wall was partly or completely obliterated. There was a crater-like elevation with heaped-up margins and excavated center.

FIG. 2. Neoplastic involvement of the epicardium and myocardium. Narrowing of the left atrioventricular orifice with calcification of the mitral valve and thickening and calcification of the chordae tendineae.

FIG. 3. Neoplastic nodules in the kidney from lymphosarcoma of the stomach.



1



2



3

FIGURES 1—3

genologic examination. A tuberculous lesion causing a mass in the wall of the stomach would be most unusual. It is not possible to narrow down the diagnosis further than to say that the patient had a malignant neoplasm involving the stomach. In such a case exploratory laparotomy is certainly indicated. Lymphosarcomas or sarcomas of other types frequently are inoperable. In this case, at the time of operation extension to the spleen and metastasis to other parts of the peritoneal cavity had occurred, and it was not feasible to attempt excision of the growth. The neoplasm involved the cardia and the patient's condition was so poor that a gastrostomy was not warranted. Gastrostomy in lesions of the cardia does not appreciably prolong life nor does it relieve the suffering of the patient. My conclusion is that this patient had a malignant neoplasm probably primary in the stomach and probably a sarcoma, and that he died of pneumonia following the exploratory operation."

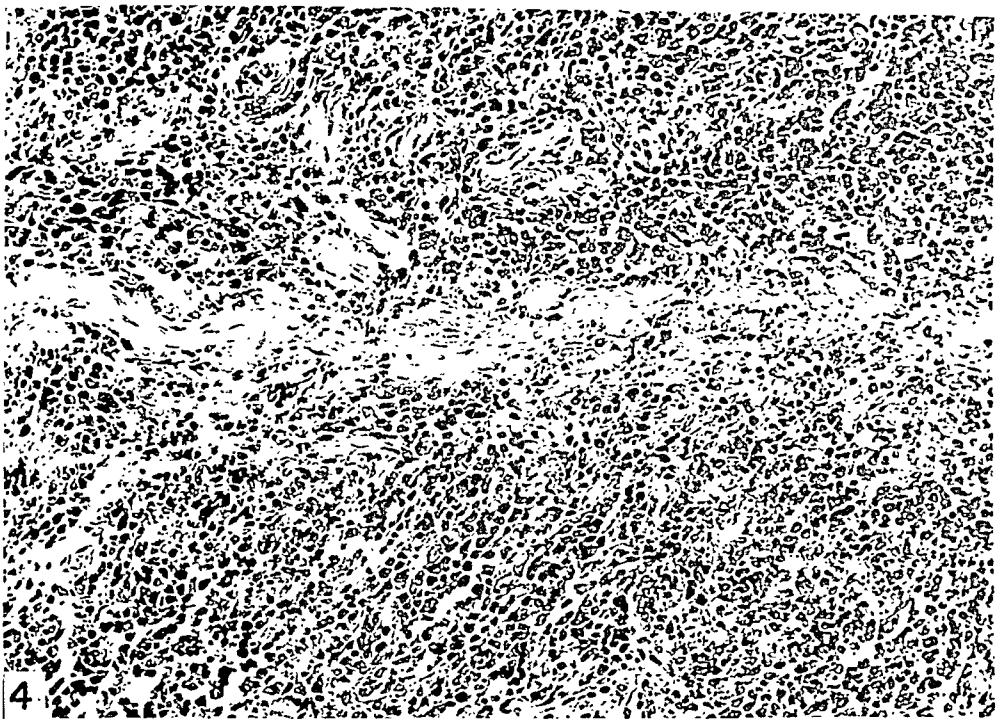
NECROPSY FINDINGS

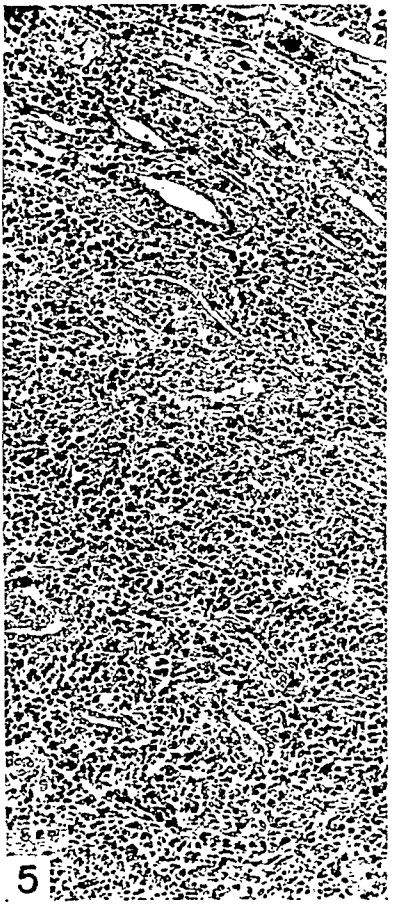
Dr. Halpert. "At necropsy there was evidence of loss of weight. The musculature of the extremities was wasted, and there was pitting on pressure of the feet and ankles. The peritoneal cavity contained about 250 cc. of blood-tinged fluid. In the left pleural cavity there was 2500 cc. of similar fluid with a specific gravity of 1.025. The right pleural cavity contained 250 cc. and the pericardial cavity 35 cc. of fluid. All these surfaces were glazed by neoplastic tissue causing adhesions between adjacent surfaces. There were also raised plaques of neoplastic deposit. The entire *stomach* up to 4 cm. proximal to the pyloric end was involved by a neoplastic process causing thickening of the wall up to 2.5 cm. along the greater curvature (Fig. 1). The neoplastic tissue was gray-white with a rubbery consistency. On the posterior wall there was a crater-like elevation 5 cm. in diameter with the margins heaped up and an excavation in the center 2.5 cm. deep. The mass was 14 cm. in diameter and, apparently by direct extension, had involved the diaphragm, the liver, the spleen and the left adrenal gland. There was enlargement and neoplastic involvement of the mesenteric and retroperitoneal lymph nodes. The *left lung* was collapsed, lumpy, and weighed 560 Gm. The *right lung* weighed 1220 Gm. and on section disclosed gray-red areas of consolidation. The *heart* was not enlarged and weighed 280 Gm. In addition to involvement of the epicardium, neoplastic tissue also infiltrated the myocardium, (Fig. 2). There was narrowing of the left atrioventricular orifice, with calcification of the mitral valve, and thickening and calcification of the chordae tendeneae extending to the papillary muscles. The aortic valve was stiff, the cusps thickened, the commissures fused, and the orifice narrowed. The wall of the right ventricle was 0.8 cm. thick. The wall of the *gallbladder* was thickened, the mucosal pattern was preserved, and the lumen contained some facetted calculi. There was an area of thickening in the fundus. On microscopic examination

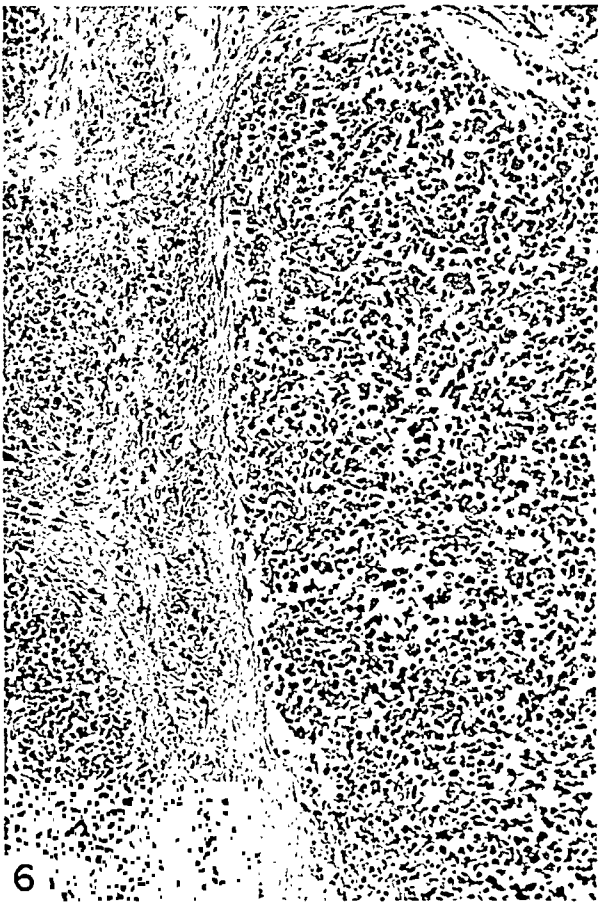
FIG. 4. Neoplastic infiltration in gastric mucosa and submucosa by large lymphoid cells. × 200.

FIG. 5. Infiltration of neoplastic cells between renal tubules and glomeruli. × 100.

FIG. 6. Involvement of the spleen, apparently by direct extension. The neoplastic cells are piled on the capsule and infiltrate the subjacent splenic tissue. × 100.







FIGURES 4-6

this had the appearance of an adenomyoma: acinar tubular structures, lined by cuboidal or columnar cells in a scanty connective tissue stroma between delicate or coarse, interlacing, smooth muscle bundles. Such an adenomyoma is the anlage either of a part of the fundus which did not develop or of the undeveloped fundus of a bifid or bilobed gallbladder. Both *kidneys* contained nodular areas of tumor which measured up to 3 cm. in diameter and produced some distortion of the calices (Fig. 3). The *prostate* was slightly enlarged, and there were slight trabeculations of the urinary bladder with two diverticula, each 1 cm. in diameter."

COMMENT AND ANATOMIC DIAGNOSIS

The edema of the feet and ankles with the accumulation of fluid in all the serous cavities was caused by a combination of nutritional deficiency with hypoproteinemia, by interference with the flow of lymph as the result of the neoplastic involvement, and by the terminal pneumonia.

The valvular changes in the heart confirmed the history of rheumatic disease. The gross and microscopic changes in the lungs were those of pneumonia which was the actual cause of death. There were also scattered areas of neoplastic involvement in the lung parenchyma as well as in the pleura.

The microscopic structure of the neoplasm as revealed by biopsy was that of a lymphosarcoma. The component cells were two to four times as large as ordinary lymphocytes, had round or oval, deeply stained, compact or reticulated nuclei and a barely discernible cytoplasm. The stroma was fibrillar, scanty, or not evident. There was a striking uniformity and lack of pleomorphism of the cells. The cellular patterns and mode of infiltration were similar at all sites of involvement, in the stomach (Fig. 4), in the spleen (Fig. 5), in the kidneys (Fig. 6), in the myocardium and elsewhere. The gross distribution of the neoplasm strongly suggested that it originated in the wall of the stomach which was extensively involved. Thence, the growth spread by direct extension to the spleen, diaphragm and peritoneal surfaces. The spread elsewhere may have been by further direct extension, or by means of lymph channels and by the blood stream. There is no reason to assume that local foci of lymphatic tissue were activated to neoplastic proliferation in any of the sites of involvement. Like the tissues of other organs, the lymphatic tissues, when involved, seemed to be invaded and replaced rather than stimulated. The final anatomic diagnosis was:

Wound of recent operation: exploratory laparotomy and excision of portion of neoplasm of stomach for biopsy. Lymphosarcoma of stomach with involvement of spleen, liver, adrenal glands, pancreas, kidneys, heart, lungs, peritoneal, pericardial and pleural surfaces and mesenteric, retroperitoneal and mediastinal lymph nodes. Hydrohemothorax, left; hydrothorax, right; hydropericardium and ascites. Emaciation with edema of ankles. Bronchitis, bronchiolitis and pneumonia, focal, bilateral. Healed mitral and aortic valvulitis with calcification and stenosis. Sclerosis of aorta with calcification. Cardiac hypertrophy, right ventricle. Cholecystitis, chronic, with cholelithiasis (mixed gallstones). Adenomyoma of fundus of gallbladder. Hyperplasia of prostate with hypertrophy and diverticula of urinary bladder.

SELECTED ABSTRACTS

Determination of Circulating Red Blood Cell Volume with Radioactive Phosphorus. R. T. NIESET, B. PORTER, W. V. TRAUTMAN, JR., R. M. BELL, W. PARSON, C. LYONS AND H. S. MAYERSON. Am. J. Physiol., **155**: 226–231, 1948.

Comparison of Results of Measurement of Red Blood Cell Volume by Direct and Indirect Technics. H. S. MAYERSON, C. LYONS, W. PARSON, R. T. NIESET AND W. V. TRAUTMAN, JR. Am. J. Physiol., **155**: 232–238, 1948.

In the first paper the authors describe the technic for studying the total blood volume, using radioactive phosphorus in the labelling of a small sample of the patient's blood. Since the amount of isotope is small and represents $\frac{1}{10}$ of the safe tolerance dose, and with 75 per cent excretion of the material in 48 hours, there is no apparent danger. The test is simple and involves the addition of 50 microcuries of P^{32} to 10 ml. of blood, incubation for two hours, and re-injection of the labelled blood into the patient. The blood injected and a sample removed 10 minutes later are counted, a hematocrit reading made and, by a simple formula, the blood volume determined. The various sources of error are discussed but they are not of any great significance. In the second paper a comparison of this method with the standard Evans blue (T-1824) technic is studied and the results tabulated. It was found that the isotope technic compared favorably and both were in agreement. The authors emphasize the importance of a small correction factor in hematocrit reading because of trapped plasma. When this is taken into account, consistent results with P^{32} can be obtained with repeated tests.

Brooklyn, New York LEO M. MEYER

Advances in our Knowledge Concerning the Etiology and Treatment of Hematological Dis orders. C. C. STURGIS. Bull. N. Y. Acad. Med., **25**: 84–99, 1949.

This paper was read before the Graduate Fortnight of the New York Academy of Medicine in 1948 and presents an up-to-date review of recent advancements in our knowledge of etiology and treatment of the anemias and lymphoblastomas. The author subdivides the anemias into three groups and describes reasons and methods for treatment. The management of agranulocytosis and leukemia includes a discussion of the value of antibiotics and folic acid antagonists. Urethane, nitrogen mustard, P_{32} and total body irradiation are categorized with proper emphasis given to each modality. Vitamin B_{12}, the animal protein factor of bacterial origin and the management of pernicious anemia patients sensitive to liver extract, are discussed. A bibliography of recent literature pertaining to all the subjects is appended.

LEO M. MEYER

The Laboratory Diagnosis of Sickle Cell Anemia with Special Reference to the Diagnostic Parameter. H. M. PERR. Blood **4**: 179–185, 1949.

Although sickle cell anemia has been recognized since 1910, no pathognomic test has been devised to distinguish it from sicklemia. The most common method of detecting the trait is to reduce the oxyhemoglobin of the red blood cell and produce sickle cells from normal biconcave discs. Utilizing the effect of carbon dioxide to produce sickling, Burch and Winsor devised the "diagnostic parameter" which was the difference between the sedimentation rates of oxygenated blood and that exposed to carbon dioxide (according to the Wintrobe method). A value of 27 mm. per hour was declared to be diagnostic of acute sickle cell anemia.

Using their technic with several minor modifications, this test was performed 307 times on 250 patients. In addition, all cases were studied with one or more of the commonly used sickling preparations; histories were taken, and physical examinations and necessary laboratory examinations were performed when indicated. Ten patients (4 per cent) had a

parameter of 27 mm. per hour or greater, but only one had acute sickle cell anemia. Fifteen patients (6 per cent) exhibited sickling, 8 of whom had a parameter of 27 mm. or more per hour.

On the basis of this data, it was concluded that the "diagnostic parameter, while highly indicative of the presence of sicklemia, does not offer a means of differential diagnosis between sickle cell anemia and sicklemia *per se*."

LEO M. MEYER

Multiple Myeloma. Its Clinical and Laboratory Diagnosis with Emphasis on Electrophoretic Abnormalities. W. S. ADAMS, E. L. ALLING AND J. S. LAURENCE. Am. J. Med., **6:** 141–161, 1949.

This paper is based on data obtained from 61 patients with multiple myeloma. The clinical observations noted the frequency of poor dentition; the high incidence in the male over 50 years of age; bone and muscle pain; weight loss; gastrointestinal complaints; fever, pallor and bleeding; and neurological sequelae. Less frequently an enlarged liver, or spleen or a bone tumor can be felt. Among the laboratory findings alterations of the electrophoretic pattern of the plasma proteins were noted in 100 per cent of patients. The authors describe these changes in great detail and present many figures to demonstrate the differences. Other frequent laboratory changes observed were the marked plasmacytosis of the bone marrow; anemia; bone changes on x-ray; hyperproteinemia with hypo-albuminemia and hyperglobulinemia; rouleaux formation of erythrocytes; renal insufficiency with azotemia; elevated alkaline phosphatase in blood; and Bence-Jones proteinuria. The authors describe the "myeloma cell" in detail and indicate its close relationship to the plasma cell. They describe the most frequent x-ray finding to be a widespread osteoporosis of the skeleton which is in accord with the recent observations of this reviewer. Another interesting suggestion is the development of a bright blue color to Wright-stained peripheral blood smears, thought to be due to the hyperproteinemia. A short discussion on differential diagnosis and treatment is included.

LEO M. MEYER

Experiments on Histamine Refractoriness. III. The Effect of Histamine Pretreatment on the Establishment of Active Sensitization. LAURENCE FARMER. J. Allergy, **19:** 358–360, 1948. *IV. Histamine Pretreatment in Eggwhite-Sensitized Guinea Pigs.* J. Allergy, **19:** 361–364, 1948.

Guinea pigs were sensitized by normal horse serum and then given repeated intraperitoneal injections of histamine acid phosphate. They were then bled and the serum was used to sensitize normal pigs passively. The latter were injected with normal horse serum 24 hours later.

The same method was used, substituting histamine azoprotein for histamine. The number of deaths was approximately the same among pigs that had received no protective injections, and those that had been injected with histamine and histamine azoprotein.

Somewhat similar experiments were carried out sensitizing the animals with eggwhite and use of the uterine strip to determine sensitivity. Pretreatment with histamine and histamine azoprotein failed to decrease the anaphylactic response to the egg.

Dallas, Texas HARVEY BLACK

Studies of Fixed Rabies Virus Propagated in the Brain of Guinea Pig Fetus. CHEN CHEN-JEN AND SAMUEL H. ZIA. J. Immunol., **60:** 17–22, 1948.

Fixed rabies cultivated in the developing fetal brain of the guinea pig *in.utero* may be put through repeated passages without loss of virulence. It is fully antigenic and makes a vaccine comparable to that of infected adult brain tissue. The advantages of this method are that fetal brain tissue is nonantigenic; that the method is very simple and no special skill is required to master the technic; the chance of contamination is very slight. It is

suggested that fetuses of larger animals might be used so that larger amounts of vaccine could be produced.

HARVEY BLACK

Study on the Mechanism of Dermatitis Venenata in the Guinea Pig with a Demonstration of Skin-Sensitizing Antibody by Passive Transfer. SEYMOUR B. CREPEA AND ROBERT A. COOKE. J. Allergy, **19**: 353–356, 1948.

Guinea pigs were sensitized to poison-ivy extract by repeated application of the antigen to the skin. Injections were then made into normal pigs using the serum of the sensitized pigs, cells expressed from the spleen and the wash water from the last washing of the cells. Sensitivity of the skin to poison-ivy antigen could be shown in 16 of the 19 that received the splenic cells. Seven of 15 receiving injections of serum gave slight skin reactions. The reaction from the washwater was slight in two. The antibody did not pass through the placenta.

HARVEY BLACK

The Determination of the Optimal Hemolysis Level for Use in the Complement-Fixation Test. JAMES W. FISHER. J. Immunol., **60**: 85–90, 1948.

Use of the logistic function for determining the amount of complement required to produce 50 per cent hemolysis has made available a method for assaying the activity of hemolysis other than in terms of minimal hemolytic dose. When these units are plotted against the various levels of hemolysin on ordinary coordinate paper, a hyperbolic curve results from which the optimal concentration of hemolysin is chosen as "that beyond which further increase fails to enhance appreciably the hemolytic activity of complement."

A modified spectrophotometric method of complement assay is used to determine objectively the optimal level of hemolysin. The curves of hemolysis at a number of levels are described by a family of linear regression lines that have a common origin but differ in slope. The optimal level is that dose which produces the maximum sensitivity of erythrocytes to complement as shown by the steepest line.

HARVEY BLACK

Gutartiger Riesenzelltumor mit Maligner Entartung nach 4 Jahren und Osteoarthropathic Hypertrophiante Pneumique durch Lungenmetastase. F. KOLLBRUNNER. Oncologia, **1**: 153, 1948.

The difference of opinion on the true character of giant cell lesions of bone continues with the contribution of Kollbrunner, a radiologist, on "Benign giant cell tumor with malignant degeneration after four years and with pulmonary osteoarthropathy by lung metastasis". In 1935 or 1936, a gardener (born 1900) injured his left leg and had difficulty in walking for three months. A similar accident with comparable results occurred in 1937; this time the leg progressively swelled. In November 1939 there was x-ray evidence of giant cell tumor in the left fibular head which was then excised; microscopic examination confirmed the diagnosis. Roentgenotherapy was then instituted.

A post-operative fistula did not close for nine months, although the patient was otherwise well and there was cortical regeneration. Swelling and pain returned, and in July penetration of the bony capsule by tumor was recognized. In November 1939, just four years after the original operation, the upper end of the left fibula was removed and the pathologist reported "sarcomatous degenerated brown giant cell tumor." July 1944, there were no roentgenologic thoracic changes, and from August to September the leg was again x-radiated. All this time the man used crutches but later became bed-ridden. In January 1945, an ulcer developed at the site of the tumor, and right bronchopneumonic foci were identified. The left leg and lower third of the thigh were amputated in May, with good healing. The patient had been complaining, however, of joint pains and swelling of hands and feet for about six months. Late in January 1946, there was a radiologic broad thoracic shadow, and characteristic changes in hands and feet of pulmonary osteo-

arthropathy. Death came May 1947. A large polymorphous cellular sarcoma of the left lung was found, with compression and shift of the mediastinum to the right.

The author states that only two-thirds of giant cell tumors remain benign, while the other one-third becomes malignant and is resistant to all treatment. He, therefore, suggests careful follow-up of all such lesions with frequent x-ray and clinical examinations. For him, roentgenotherapy remains the treatment of choice.

Fort Wayne, Indiana S. M. RABSON

Ginecomastia con Degenerazione Cancerigna in Prostatico dopo Trattamento Estrogeno. GIOVANNI F. GARDINI. Oncologia, **1**: 129, 1948.

The Italian title, "Gynecomastia with cancerous degeneration in a prostatic [patient] after estrogenic treatment", fails to indicate that the neoplasm appeared in the breast, or that the patient suffered from carcinoma of the prostate. A 60 year old man was first seen in July 1945 with what was diagnosed clinically as prostatic carcinoma (cystoscopy was not recorded), and roentgen therapy instituted. A year later there was evidence of osteoplastic vertebral metastasis, and also enlargement of supraclavicular, lateral cervical, and axillary lymph nodes. A node (site not stated) was excised and Businco reported secondary carcinoma, which Gardini, a member of the department of radiology at the University of Bologna, regarded as metastatic from the prostate. X-ray therapy was again applied. August 1946 the lymph nodal status was unchanged. Cystoscopy uncovered a circumscribed vegetative lesion at the urinary bladder trigone, especially pronounced on the right side. Rectal examination suggested that the prostate, rather than the bladder was primarily attacked. Estrogen treatment was begun. In December 1946, the man was greatly improved, the prostate better delimited and somewhat softer, and the vesical newgrowth had disappeared.

In February 1947, vesical and urinary status was unchanged, but lymph node enlargement had reappeared; biopsy was done, and the microscopic picture was similar to that of July 1946. Estrogen therapy was continued in the same dosage (6 mg. daily) but with a different preparation, and another course of x-ray treatment carried out.

In May 1947, there was no adenopathy, but both breasts were enlarged. The vertebral lesion, formerly seen in the thoracic area, was now also noted in the lumbar region, pelvis, and left femur. Two months later (July) there was a tumor in the left breast, and that organ was removed. Grossly, the neoplasm suggested cancer, and, microscopically, Businco described an "infiltrating neoplasm of epithelial nature, most probably secondary". The photomicrographs of the two biopsy specimens and of the mammary newgrowth are much alike.

In the months which followed, there was urinary suppression (the vesical lesion had reappeared), increasing submaxillary lymph node enlargement, and the appearance of neoplasm in the operative scar. A few days before death (October 1947), much of the skeleton was riddled by osteoplastic metastasis.

At necropsy carcinoma was found in the bladder, presumably of vesical origin. There was infiltration of the pelvic tissues, and metastasis to peritoneum, to aortic, mediastinal and cervical lymph nodes, to bones, to both adrenal glands, and to the skin of the left breast. Microscopically, normal prostatic tissues were not identifiable; the organ was entirely replaced by newgrowth. Gardini regarded the tumor as intrinsic prostatic cancer.

As for the mammary tumor he considered it primary in the breast, provoked, as was the gynecomastia, by the hormone treatment. Examination of the photomicrograph of the breast cancer, however, tends to support the view that that new growth was metastatic from the prostatic carcinoma.

One observation of Gardini is worth recording, namely, that metastases present before the initiation of estrogen therapy became, for a short time, less radioresistant.

S. M. RABSON

Metastasierender Hypernephroider Nieren-tumor mit Ungewöhnlich Langem Krankheitsverlauf. CH. BOTSZTEJN AND H. U. ZOLLINGER. Oncologia, **1**: 165, 1948.

At the age of 57 years a woman noted difficulty in swallowing, a faint sense of weakness, and slight weight loss. Iodine therapy was fruitless and one-half year later (September 1939) she entered the hospital. There was a diffuse struma with tracheal compression. In the x-ray film there was evidence of large nodular metastasis in both lungs. This was assumed to be secondary to thyroid carcinoma, and neither operation nor roentgenotherapy was attempted. One-half year later, because of increasing respiratory difficulty, partial thyroidectomy was performed. Despite the metastatic state the general condition was good, and the patient went home after a week. Microscopically, in the thyroid specimen, there was no evidence of anaplasia. Many cells were foamy, and the diagnosis was "struma carcinomatosa, in part solid simple, and in part large cellular, small alveolar (Getzowa)".

For six years the woman remained free of symptoms and fully well, even though the size and number of the lung metastases increased; there was no recurrence of the struma. Early in 1946, however, there was increasing pain in the left leg caused by metastasis in the great trochanter. X-radiation was given in July 1946. Four months later, the femoral neoplasm was unchanged, but the pulmonary metastases were more prominent. Death came in January 1948, with evidence of right-sided heart failure.

At necropsy, the primary neoplasm, a hypernephroid carcinoma, was found in the left kidney, and secondary growths in the anterior mediastinum, lungs, left femoral neck, and thyroid. It was recognized that the neoplasm in the resected portion of thyroid, too, was metastatic renal cancer. The long period of life with neoplasia is attributed to "stagnation on a relatively lower level of malignancy in the theory of Apitz" (Apitz, K.: Virchows Arch. f. path. Anat., **301**: 593, 1943).

S. M. RABSON

Ein Nickhautcarcinom bei einem Hirsch. RENÉ BERNOULLI. Oncologia, **1**: 178, 1948.

Bernoulli adds another report to the growing literature on neoplasia in wild animals. A papillomatous cystic carcinoma is described in the nictating membrane of an old deer hind.

S. M. RABSON

Beiträge zur Rolle des Follikelhormons bei der Entstehung des Gebärmutterschleimhautkrebses. L. VON VÁCZY. Oncologia, **1**: 183, 1948.

"In a review of ten years of operative material at the II. Gynecology Clinic, Budapest, 8 cases of endometrial carcinoma, 2.1%, were found among 364 cases of glandular-cystic hyperplasia. The appearance of cancer in glandular-cystic hyperplasia in so high a number supports the concept of those investigators who ascribe a role to follicle hormone in the appearance of genital carcinoma." (Author's summary.)

Sippentafel mit Gehäuftem Krebsvorkommen. UMBERTO COCCHI AND PETER STUECKELBERGER. Oncologia, **1**: 143, 1948.

Cocchi and Stueckelberger very cautiously introduce "Ancestral Tree with Frequent Appearance of Cancer" by remarking that the technically uninitiated tend to regard the frequent appearance of cancer in a single family as evidence of an inherited disposition. Such a state can be completely accidental, especially in Switzerland where 16% of deaths in recent years are ascribed to malignant neoplasia. Bashford (Bashford, E. F.: Deutsch. med. Wchnschr., 4, 1913) is quoted on the probability of cancer in 100 families, each with 6 members, all over 35 years of age, and in a population where 10 per cent of deaths are caused by tumors. Fifty-one families will be free, 36 will have one or more cases, 11, two or more, and 3, three or more. Climate, habits of life, customs, and occupation, also play their parts, if not in direct causation, at least as predisposing or exaggerating factors.

The authors point out that it is not possible to separate "environmental" from hereditary cancer, that "every characteristic of a phenotype is the reaction to external stimuli of one or more genes in the genotype", and that cancer, as such, is not hereditary but is a phenomenon of the phenotype. Heredity in cancer may be studied by family tree, investiga-

tion of twins, by mass statistics, and by ancestral investigation; the advantages and disadvantages of each method are evaluated.

The study begins with a 66 year old woman with gastric carcinoma, who stated that cancer was especially common among her relatives. She was one of 8 siblings, of whom 2 were alive; one of the other 4 died of neoplasia (gastric). A cousin had tongue cancer, and of 7 uncles, aunts, father and mother, all dead, 6 had passed away with cancer (including 3 gastric and 2 intestinal). Of 4 grandparents, 2 were known to have perished with new-growths. In short, of 21 of 24 persons about whom reliable information was known, 10 had cancer.

With this as a basis, the authors then investigated numerous branches of the family, and found 296 persons in 8 generations; information was available concerning 281. Of 281, 17 (6 per cent) had cancer: stomach, 12; pancreas, 1; rectum, 1; tongue, 1; larynx or esophagus, 1; uterus, 1. None was found under 40 years old; of the 98 living or dead, over 40 years, 17.3 per cent, and of the 131 over 30 years, 13 per cent, were attacked by neoplasia. If the statistics are concerned only with the dead members of the group, 32.7 per cent of those who died at or over 40 years of age succumbed to cancer. This high percentage is more than double the calculated value for Swiss over 40 years old for the years 1931 to 1941; this status is also true for the group over 30 years of age.

Cocchi and Stueckelberger conclude by remarking that "with this accumulation of malignant tumors we can speak of a *cancer group* [clan] analogous to the concept of cancer family. This is an exceptionally rare occurrence, because, among our 168 groups, we have only thrice encountered a similar cancer accumulation, which justifies us in regarding a group as a cancer group."

S. M. RABSON

Die Lebensfähigkeit von Mycobacterium tuberculosis in Schweizerischen Käsesorten. P. KÄSTLI. Schweiz. Ztschr. f. Path. u. Bakt., **11**: 408, 1948.

In this somewhat disturbed world, other nations must export to secure dollars with which to purchase our products. Among Switzerland's exports is Swiss cheese, for which we are a good customer. The current opinion in America holds that all milk, including that for cheese-making, be pasteurized. The Swiss find, however, that they cannot produce a hard cheese of high quality with pasteurized milk, hence, a dilemma which Kästli has attempted to resolve. Guinea pig-inoculation experiments in which the animals are injected with emulsions of cheese made with tubercle bacillus-infected milk indicate that negative results are consistently obtained after the cheese is a month old. The reasons for this sterility may include the low water content of the cheese, as well as the presence of bactericidal or antibiotic substances early in the manufacture. Tison (Ann. Inst. Pasteur, 1947) described bactericidal effects of proteolytic microorganisms, and Mattick and Hirsch (Lancet, 1946), antibiotic action of some lactic acid streptococci on the tubercle bacillus. Swiss cheese manufacture may offer a fertile field for streptomycin competitors.

S. M. RABSON

Die Eiweizsstoffwechselstörungen des Plasmocytomkranken. K. A. BRASS. Frankf. Ztschr. f. Path., **59**: 413, 1948.

Brass has previously reported on the morphology of protein metabolic disturbances in myeloma (Frankf. Ztschr. f. Path., **57**: 367, 481, 1943; **58**: 56, 1943). In the present communication he describes 7 cases of myeloma in 3 of which pathologic protein crystals, "para-proteins" (Apitz, K.: Virchows Arch. f. path. Anat., **300**: 113, 1937; **306**: 631, 1940), were identified in the plasmocytes. Apitz considers the presence of the crystals as an expression of protein secretion but Brass regards them as indicating metabolic disturbance in which the cells are unable to elaborate the metabolic building blocks proffered them.

In the 4 other cases Brass saw the crystals or "colloid-crystals" outside the plasmocytes.

In one instance in which only the bone marrow was studied, the material was found in the reticulum, but the tumor cells were free of the material. In another, the Kupffer cells of the liver and the splenic venous endothelium were affected. The third case involved the endothelium of the small veins of the spleen, as well as the plasmocytes. In the fourth subject, not only the splenic endothelium, but also renal tubules, stromal cells, interstitial leukocytes, and even lymphatic endothelium sheltered the abnormal protein.

With Apitz, Brass regards Russell's bodies as evidence of the "plasma cell nature of myelomas". In addition, he has seen the bodies as inclusions in plasmocytes in quantities not ordinarily found in plasma-cell infiltrates.

The author also devotes attention to the role of the kidney in myeloma. Of the 5 of 7 cases in which there were both adequate history and anatomic material, death was caused by uremia in 3 instances. Unlike Ehrich (Ztschr. f. klin. Med., **121:** 398, 1932), who attributed renal insufficiency to mechanical plugging of renal tubules by casts, Brass joins Nonnenbruch (Deutsch. Arch. f. klin. Med., **189:** 56, 1942) in ascribing the cause of renal failure to extrarenal factors. He reserves discussion of interstitial nephritis (edema, infiltration with lymphocytes, plasma cells, and leukocytes) so frequently encountered, for a later report.

S. M. RABSON

Circulating Anticoagulant as a Cause of Hemorrhagic Diathesis in Man. C. LOCKARD CONLEY, HOWARD K. RATHBUN, WILLIAM I. MORSE, II, AND JAMES E. ROBINSON, JR. Bull. Johns Hopkins Hosp., **83:** 288–296, 1948.

Most hemorrhagic syndromes are associated with defective blood coagulation attributed to a deficiency of one of the components necessary for the mechanism of the coagulation system. The authors studied three patients in whom a hemorrhagic syndrome resulted from the spontaneous occurrence of an anticoagulant in the circulating blood.

Blood was withdrawn from each of the three patients using silicone-treated needles, syringes and tubes. The supernatant plasma, after centrifugation, was essentially platelet-free and failed to clot even when stored for days in glass tubes. Small amounts of these plasmas acted as a powerful anticoagulant when added to freshly drawn normal human blood. In the first two cases, the anticoagulant present was of such titer that one part of the patient's plasma in 100 parts of normal blood prolonged the clotting time of the latter to an appreciable extent. Toluidine blue and protamine (known heparin antagonists) were without significant effect on the clotting time of these patients. The anticoagulant was not destroyed by dialysis against normal saline solution or by heating to 65 C. for five minutes. The three anticoagulants appeared to act at different stages in the coagulation system.

The literature of similar cases is reviewed, and the authors emphasize the necessity for adequate anticoagulant assays before placing such patients in the diagnostic category of "pseudo-hemophilia" or similar conditions.

Cleveland BEN FISHER

Further Studies On the Gram Stain. HERMAN BAKER AND WALTER LYNN BLOOD. J. Bact., **56:** 387–390, 1948.

The theory of the Gram stain is reviewed, beginning with the earliest ideas and chronologically proceeding through the biochemical-bacteriologic studies of the past five years. It is noted that the Gram-staining characteristics of organisms may be changed by altering the physical and biochemical conditions of the substrate.

The authors' own study concerns production of Gram-positiveness in cultures of *Escherichia coli* by the addition of a highly viscous preparation of desoxyribonucleic acid. The Gram-positiveness could be washed away with distilled water, but not with physiologic saline solution.

BEN FISHER

Inconstancy and Variability of the Vascular Fragility Test Even in Purpuric Conditions. J. Roskam, Ch. Renard, and L. Swalue. Blood., **3:** 1112–1119, 1948.

The association of both hemic and vascular factors in the production of purpura is reviewed. Using the "venostasis" type of vascular fragility test, the authors noted that the hemorrhagic lesions which were produced had a qualitative and a quantitative significance. Petechiae which had a diameter greater than 1 mm. were more often associated with classical hemorrhagic disease, and were observed to parallel an increased bleeding time.

Two cases of "purpura simplex" and a case of thrombocytopenic purpura are reviewed and the inconstant results of the vascular fragility test in these three cases is demonstrated. However, the authors are rather vague as to the possible etiology of the purpura in the first two cases (hypersensitivity?). This dissimilarity in etiology may account for the variable results in their observations.

Ben Fisher

The Value and the Limitations of the Coagulation Time in the Study of the Hemorrhagic Diseases. Armand J. Quick, René Honorato C., and Mario Stefanini. Blood, **3:** 1120–1129, 1948.

This interesting paper attempts to minimize the empirical status of the coagulation-time test and highlights the advantages and disadvantages of this procedure as applied in a practical manner. The significance of the test is discussed in light of the recent discoveries of new coagulation factors, and of new information concerning the mechanism of the clotting defect in various clinical conditions, especially hemophilia.

It is emphasized that the chief value of the coagulation test is in its screening of possible prolonged bleeding. However, further study of other factors is then indicated to classify the defect properly. Quick's prothrombin consumption test is also discussed.

Ben Fisher

A Method of Staining Nucleoli of Cells in Fresh Benign and Malignant Tissues. W. W. Ayres. Cancer Research, **8:** 352–360, 1948.

The increasing utilization of desquamative cytology for cancer diagnosis puts a premium on any method by which distinctive features might be demonstrated in isolated malignant cells. The author attempts to utilize for such a purpose supravital staining of nucleoli in fresh tissues, transudates and exudates. Azure C, a methylene blue derivative, is used in 0.25 or 0.5 per cent solution in methyl alcohol. Microslides are stained with the solution which is permitted to evaporate. A suspension of the cells to be examined in human serum is prepared on a coverslip which is then inverted over the stained slide. Within one to 15 minutes the slides can be read. The nucleoli stain a uniformly dark azure, while nuclei do not stain at all, or only faintly. The structural details of nucleoli in a number of normal blood and tissue cells are described, and contrasted with those of malignant cells (10 photomicrographs). In cancer cells, evidence of nucleolar division and of loss of polarity was frequently noted, although these changes are not strictly pathognomonic. Nevertheless, the author has found the method of supravital nucleolar staining useful and reliable in about 200 cases. He calls special attention to the help derived from the method in distinguishing in body fluids mesothelial cells and macrophages from cancer cells. He also proposes to use the stain in conjunction with frozen sections in order to visualize cytologic details.

Chicago Kurt Stern

BOOK REVIEWS

Handbook of Practical Bacteriology. A Guide to Bacteriological Laboratory Work. Ed. 8. T. J. MACKIE, C.B.E., M.D., LL.D., D.P.H., Professor of Bacteriology, University of Edinburgh; Honorary Bacteriologist to the Royal Infirmary, Edinburgh; Director of Bacteriological Services, City of Edinburgh; and J. E. MCCARTNEY, M.D., D.Sc., Director of Research and Pathological Services, London County Council; Fellow of the Rockefeller Institute for Medical Research, New York. 624 pp. $7.00. Baltimore: The Williams & Wilkins Company, 1948.

This American edition of a text familiar for years to students and laboratory workers in England should be a welcome addition to the bacteriological library. Among American texts, *Diagnostic Bacteriology* by Schaub and Foley, is perhaps the nearest equivalent to "Mackie and McCartney", although it is not nearly so comprehensive in its subject matter.

Part I of Mackie and McCartney deals with the general biology of microorganisms and with immunity in relation to practical bacteriology. Part II is concerned with bacteriological technic, while Part III is a highly condensed discussion of pathogenic and commensal microorganisms, including filterable viruses and rickettsiae, together with the application of this information in bacteriological diagnosis. The subject matter has been brought up to date on such items as the testing of antibiotics, the Kahn verification test, electron microscopy and fluorescence.

In the opinion of the reviewer, this is an unusually well written, usable text for the student or practical worker. The information on both theory and procedures is clear and up-to-date. A happy balance has been maintained in the presentation of theory and in its application. Statements are concise but readily grasped by the worker possessing limited knowledge of the field. The discussion of commensal organisms which accompanies each group of pathogens is of considerable value; it points out possible sources of error to the inexperienced worker.

Because of differences in the source of media and reagents, as well as differences in laboratory practices, an American reader frequently must "translate" the text into more familiar terms or products. There is evidence of the lack of certain precision instruments which we take for granted, such as the use of indicators rather than a pH meter. Such differences will perhaps add, rather than detract from the interest American workers experience in this text.

Lansing, Michigan G. D. CUMMINGS

Zinsser's Textbook of Bacteriology. Ed. 9. Revised by DAVID T. SMITH, M.D., Professor of Bacteriology and Associate Professor of Medicine, Duke University School of Medicine. By DONALD S. MARTIN, M.D.; NORMAN F. CONANT, Ph.D.; JOSEPH W. BEARD, M.D.; GRANT TAYLOR, M.D.; HENRY I. KOHN, M.D.; and MARY A. POSTON, M.A., all of Duke University School of Medicine. 992 pp., 251 figs. $10.00. New York: Appleton-Century-Crofts, Inc., 1948.

Although the ninth edition follows very closely and with only minor variations the general outline of construction used in previous editions, the text has been entirely reset and very largely rewritten. Information is current and includes references through 1947. The first portion of the book concerns itself with the general principles of bacteriology and immunology and summarizes very well current information in these fields. The authors are to be particularly commended for their presentation, in text and illustration, of the recent information made available through the medium of the electron microscope. The portion of the text dealing with immunological principles and procedures presents a difficult subject in a very lucid and understandable way. The remainder of the text discusses infectious disease by etiologic agent, presenting as a unit the bacteriology, clinical symptomatology, epidemiology, prophylaxis and therapy. Of particular interest is the apparent ease with which the authors discuss the difficult problem of the antigenic structure of

Salmonella and Shigella. Information in reference to specific biologic products for immunization or therapy is presented in more detail than is customary in a text book of this type. In general, physicians or medical students will find adequate, and in the main, very accurate information in reference to the methods of preparation, standardization and use of biologic products. The book brings up to date current information in reference to the pleuropneumonia group of organisms, Q fever and Rickettsiapox. Virus diseases are classified and discussed on the basis of the presenting symptomatology. As one might well expect, the portion of the text on fungus diseases reflects the authors' interest and authority in this field.

This book is to be recommended highly as a text book for advanced students in bacteriology and immunology, for medical students and for physicians. Revision has been so complete that it may well be considered as entirely rewritten; information is current; coverage is adequate and in many aspects in more detail than in any other single text book with which the reviewer is acquainted.

Lansing, Michigan H. E. COPE

Surgical Pathology. By PETER HERBUT, M.D. Professor of Pathology, Jefferson Medical College, Philadelphia, Pa. 710 pp., 410 illus. $12.00. Philadelphia: Lea and Febiger, 1948.

In the preface the author clearly sets forth the aims of the book and adequately carries these out in the subsequent chapters. The book is primarily written as a ready reference for surgeons and for those preparing for specialty board examinations. It is not designed as an exhaustive treatise on all phases of surgical pathology, certain subjects, such as the brain, spinal cord and eye, being intentionally omitted. The regional approach to the subject is practical and adequate. In all chapters certain nonsurgical conditions of interest and relative importance are included and add to the usefulness of the work. The summaries of clinical findings are brief but a step in the right direction. Perhaps expansion of this idea would enhance the usefulness of the book to surgeons. The diseases discussed are frequently illustrated by photographs of gross specimens and photomicrographs of average to good quality. A few subjects are treated sketchily, notably benign tumors of the kidney, concerning which I believe urologists would want to have more information. The discussion of adrenal tumors is omitted entirely. The selected bibliography at the end of each chapter is excellent and may be quite useful to pathologist as well as surgeon. Most references are from 1935 to 1943, with a goodly number in 1945 and some in 1946 and 1947. The index is quite workable and complete.

Grand Rapids, Michigan C. ALLEN PAYNE

Techniques of Histo- and Cytochemistry. By DAVID GLICK, PH.D., Associate Professor of Physiological Chemistry, The Medical School, University of Minnesota. 531 pp., 159 figs. $8.00. New York and London: Interscience Publishers, 1949.

This new book by Glick is a most valuable addition to the literature of histochemical technic. It is the only one in English; in fact, even Lison's old French textbook is completely out of print.

The book deals in detail with all aspects of histochemistry in the wider sense of the word; both the simpler technics of direct microscopic visualization and those of emission and absorption spectroscopy, x-ray spectroscopy, analytical electron microscopy, radioautography, together with the ingenious microchemical methods developed mainly by the Carlsberg group, and procedures for the mechanical separation of cell constituents are adequately covered. The bibliography is excellent.

For the research worker in histochemistry Glick's book is indispensable and, in addition, it will be found to be a highly useful work of reference in all histologic laboratories.

Chicago GEORGE GOMORI

Introduction to Physiological and Pathological Chemistry. Ed. 3. By L. Earle Arnow, M.D., Director of Research, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pennsylvania. 595 pp., 140 figs. $4.00. St. Louis: The C. V. Mosby Company, 1949.

In the third edition of this textbook, the divisions of the previous editions are maintained, Part I, Introduction to Chemical Science, Part II, Physiological and Pathological Chemistry; Part III, Introduction to Laboratory Chemistry; and an appendix. As indicated by the title, the emphasis is on the physiologic and pathologic phases; though the essential topics of inorganic and organic chemistry make up about 45 per cent of the text, exclusive of Part III.

The book is designed primarily for nurses and should impress them with the importance of chemistry in medicine, as the emphasis in both text and illustrations is on the pathologic phases.

However, three objections may be raised. 1. In Part II, the discussion of the metabolism of each of the various tissue constituents follows the discussion of the chemistry of that component and precedes the full discussion of digestion. 2. Absence of a chapter on blood is to be regretted for without it the student may not appreciate how the blood functions through the chemical behavior of its various components. 3. With the inclusion of Part III, Introduction to Laboratory Chemistry, the instructor is either limited to the use of these experiments or the student is burdened with an unused portion of a fairly large text book and the increased financial outlay for a laboratory manual of the instructor's choice.

To the reviewer, the inclusion in the appendix of 34 pages devoted to removal of stains from fabrics seems another burden to the student. How much better it would be for the student to purchase the government bulletin from which the material in the text is taken! That pamphlet would have a better chance of being kept and used than the textbook.

The author is to be commended for his skillful incorporation of recent developments with sufficient detail to give the nurse a good background and for the excellent editorial work resulting in a book practically free of errors.

Ann Arbor, Michigan Lila Miller

Oral Anatomy. By Harry Sicher, M.D., Professor of Anatomy and Histology, Loyola University School of Dentistry, Chicago College of Dental Surgery, 529 pp., 310 figs. $15.00. St. Louis: The C. V. Mosby Company, 1949.

Some 20 years ago, the author of this book collaborated with the eminent Viennese anatomist Julius Tandler and produced a German anatomy for dentists. The present outgrowth of the author's continued concern with the teaching of dental anatomy should be of real value to oral surgeons, dentists and otologists especially.

Sicher's treatment of the anatomy of the oral and related regions is a mature and practical approach in which anatomy and its applications are well interrelated. The figures, either newly prepared or from the author's previous books, present much in a clear fashion. The index is complete. There are no references, and there is no attempt to review and evaluate the literature. This is an author-to-reader book.

The early chapters (380 pp.) present the systematic anatomy of the head and neck—osteology, muscles, blood vessels, viscera, etc.—in an adequate manner. Practical points are noted in passing and the text reads more leisurely than the big gross anatomies. Lymphatics are treated rather sketchily. The section on the skull, however, is a most excellent account—especially the pages on the regions of the skull, on skull and facial growth and on craniometry.

The last one hundred pages deal with practical problems that relate to the regional anatomy. These pages include well-handled chapters on the alveolar processes in relation to the teeth, on the anatomy of local anaesthesia, on arterial ligation and related problems, on tracheotomy, on the temporo-mandibular joint and on the fascial spaces in relation to

the spread of infection. Sicher's treatment of the fascial spaces of the neck is in part a personal interpretation with which many anatomists would not agree.

Ann Arbor, Michigan W. T. DEMPSTER

Clinical Aspects and Treatment of Surgical Infections. By FRANK LAMONT MELENEY, M.D., F.A.C.S., Associate Professor of Clinical Surgery, College of Physicians and Surgeons, Columbia University; Associate Visiting Surgeon, Presbyterian Hospital, New York City. 840 pp., 287 figs. $12.00. Philadelphia: W. B. Saunders Company, 1949.

In orderly fashion this volume presents the surgical infections of regions, organs, and tissues. Its authority is the author's twenty-five and more years of broad surgical practice combined with fundamental studies of the bacteriology of surgical diseases, and a discriminating use of the best literature. Its technic may be illustrated by the one topic, "Suppurative Pericarditis". There follows under this heading a discussion of pathogenesis, bacteriology, symptoms, signs, treatment, and two pertinent case reports. By this means one is made to appreciate the relationship of specific organisms to the entire clinical history of a disease state. One is told what organisms are suspect in a clinical situation, the importance of their identification, and then how to check or abolish their evil effect. Sulfonamides, penicillin, bacitracin, and streptomycin are critically appraised insofar as experience to date will allow, and in their relation to surgical therapy. The liberal use of illustrative case reports, many going back to the pre-antibiotic years, is most helpful. This is primarily a clinical work and intended as a companion piece to the author's more fundamental "Treatise on Surgical Infections" published last year. It is a surgeon's book, but is valuable to all who would know the relationship of bacteriology to surgical disease. The photographs, line drawings, and charts are numerous, well chosen, and nicely reproduced. The format and typography are pleasing to the reader and a credit to the publisher. There is an excellent bibliography at the end of each chapter, and a useful index closes the book.

Dearborn, Michigan GAYLORD S. BATES

Venous Thrombosis and Pulmonary Embolism. By HAROLD NEUHOF, M.D., Clinical Professor of Surgery in Columbia University, Consulting Surgeon to Mount Sinai, Montefiore, Beth El, and Hackensack, N. J. Hospitals. 159 pp., 31 figs., 12 tables. $4.50. New York: Grune and Stratton, 1948.

In recent years much new knowledge has been gained in the physiology, etiology, diagnosis, as well as treatment of venous thrombosis and pulmonary embolism. This monograph presents the results of extensive studies in nearly a hundred cases. It is apparent from this book that the answers to many questions have not yet been obtained. In the analysis of individual cases the author appears to favor anti-coagulant therapy, rather than isolation of the femoral venous segment by surgical means.

Because of the magnitude of the problem, as well as the lack of agreement concerning many of its important aspects, this book is an important one. Though it appears to have been written primarily for surgeons, other medical men, such as internists, obstetricians and orthopedic surgeons, could also well afford to give it a prominent position in their libraries.

Eloise, Michigan DARRELL A. CAMPBELL

Diagnostické nabodávání mízních uzlin, nádorů a dužinových orgánů. (*Diagnostic Puncture of Lymph Nodes, Tumors and Parenchymatous Viscera.*) By JOSEF LIBÁNSKÝ, M.D., Assistant, First Clinic of Internal Medicine, Karlovy University. 172 pp., 126 figs. Prague: Medical Bookshop and Printshop, 1948.

The major portion of this volume is devoted to the cytologic examination of puncture material from lymph nodes, while puncture of the liver and of other organs represents merely an appendix to the text.

The technic of puncture of lymph nodes is described. The topics discussed include cytologic findings in normal and hyperplastic lymph nodes; nodes in infectious mononucleosis and Hodgkin's disease; reticulo-endothelial cells of lymph nodes, spleen and bone marrow; reticulo-endotheliosis and storage diseases, histoplasmosis, coccidiomycosis, kala-azar, mycosis fungoides, sarcoidosis and malaria; various forms of leukemia, reticulum cell sarcoma, lymphosarcoma, myeloma and metastatic tumors.

The conclusions are translated into the English and French languages.

The outstanding feature of this valuable monograph is a series of 126 beautiful, black and white photomicrographs of cells obtained by the puncture of lymph nodes.

Boston O. J. POLLAK

Mikrobiologické vyšetřovací methody a stručná diagnostika v praxi. (*Microbiologic and Brief Practical Diagnostic Methods.*) By DOC. DR. IVAN MÁLEK and DOC. DR. VLADIMÍR WAGNER. 422 pp., 116 figs. Prague: Association of Czech Physicians, 1948.

This book has three parts. The first part deals with methods for obtaining material for microbiologic studies. The second part considers bacteriologic and serologic methods to be employed on material from a given source and directions for its study in specific diseases. The third part comprises the diagnostic steps for individual microorganisms, and here Bergey's classification is followed loosely.

Although the problems are attacked from all possible angles, the text is not repetitious. The book is well arranged and the script is up-to-date. The illustrations include good colored plates and photomicrographs and especially good close-up photographs of bacterial colonies.

An English translation of this book would be worth while.

O. J. POLLAK

Terezín očima hygienika. Zpráva z terezínského koncentračního tábora o boji proti hmyzu a skvrnitému tyfu. (*Terezín as Seen by the Epidemiologist. A Report from the Concentration Camp in Terezin Concerning the Campaign Against Insects and Exanthematic typhus.*) By DR. J. PICK, DR. R. POLÁK, and J. PACOVSKÝ. 173 pp., 9 figs. Prague: Medical Bookshop and Printshop, 1948.

Just before termination of the World War II, the Germans brought to Terezin 13,000 physical and mental human wrecks. Several epidemics broke out. The typhus morbidity was 2202 and the mortality 503 (22.8 per cent). The maximum number of typhus patients in a single week was 745 of whom 130 died. The typhus epidemic lasted for ten weeks.

In the light of passive resistance of the occupational authorities, the task of the epidemiologists, the authors of this book, was tremendous and the fight heroic. Hidden in a narrative of the daily plight of the camp's medical staff, which was periodically depleted through deportations and executions, is a wealth of informations documented by statistics. Most significant are the chapters dealing with the efficacy of various gaseous disinfectants for clothing, luggage, cots and barracks.

This is interesting reading for the epidemiologist.

O. J. POLLAK

The Renal Origin of Hypertension. By HARRY GOLDBLATT, M.D., C.M., Director, Institute for Medical Research, Cedars of Lebanon Hospital, Professor of Pathology, University of Southern California, Los Angeles, California. 126 pp., 37 figs., 3 tables. $2.75. Springfield, Ill.: Charles C Thomas, 1948.

This book on pathogenesis of hypertension was written by an experimental pathologist who has devoted years of careful study to the problem. It was composed as a "written lecture" and was edited by Paul R. Cannon. The text is a model for concise and conservative statement. It includes chapters on methods of producing experimental renal hypertension, on pathologic changes induced in animals and on the mechanism of development of hypertension, also chapters on renin, hypertensinogen, hypertensin, hypertensinase and

the juxtaglomerular apparatus. In the light of experimental findings and of present concepts concerning pathogenesis, the author also discusses medical and surgical treatment of hypertension and, in a final chapter, compares the findings in human essential and experimental human hypertension.

This volume should serve as an important and handy reference to all who are interested in the subject of pathogenesis of hypertension. This is an example of bookmaking in the best "Thomas tradition".

Atlas of Neuropathology. By Wm. Blackwood, M.B., F.R.C.S.E., Assistant Pathologist, The National Hospital, Queen Square, London; T. C. Dodds, F.I.M.L.T., F.I.B.P., F.R.P.S., Laboratory Supervisor Department of Pathology, University of Edinburgh, Lecturer to the Society of Radiographers, Scottish Branch; and J. C. Sommerville, A.I.M.L.T., Senior Technician, the Department of Neuropathology, University of Edinburgh and Scottish Mental Hospitals' Laboratory. 199 pp., 262 figs. $9.00. Baltimore: The Williams & Wilkins Company, 1949.

It is a delight to browse through or to study carefully any portion of this exquisitely well illustrated atlas. The subject material and figures have been selected with uncommon care and the descriptions as well as the legends are authoritative and "to the point". Of the various atlases on neuropathology that have come to the attention of the reviewer during recent years, this is unquestionably the best. Pathologists and neurologists alike will find it to contain considerable material of genuine value to them. It is recommended without reservation.

Radioactive Indicators, Their Application in Biochemistry, Animal Physiology and Pathology. By George Hevesy, Institute for Research in Organic Chemistry, University of Stockholm, Sweden; Institute for Theoretical Physics, University of Copenhagen, Denmark. 556 pp., 97 figs., 208 tables. $10.00. New York: Interscience Publishers, 1948.

This book is probably the most useful, informative and authoritative treatise that has yet been published on the application of tracer methods to physiologic processes. Dr. Hevesy was one of the early investigators in the use of tracer methods in the study of biologic phenomena and it will be recalled that in 1943 he received the Nobel prize in chemistry for his achievements in this field.

Fundamental work with radioactive indicators in the fields of animal physiology, pathology and biochemistry has been carefully reviewed, appraised and documented with reference to original investigations. Special interest for clinical pathologists will be found in sections dealing with the application of tracer technics for measuring the volume of extracellular fluids, the circulation time, cell permeability and the absorption of isotopes and their turnover in the body. It is believed that this volume will prove to be an excellent reference book for the library of physicians interested in this rapidly expanding field.

Cleveland F. William Sunderman

OBITUARIES

Frederick Boerner

Dr. Frederick Boerner died on September 16, 1948. With his passing, Philadelphia lost one of its ablest clinical pathologists. Dr. Boerner was born at Cape May, N. J., on April 27, 1889. Following his preliminary education he entered the Veterinary School of the University of Pennsylvania from which he was graduated in 1912 with the degree of Doctor of Veterinary Medicine.

From 1912 to 1923 he served as Director of the Laboratory of Animal Industry of the Commonwealth of Pennsylvania. In 1923 he relinquished this position to become Bacteriologist to the Graduate Hospital of the University of Pennsylvania. He was advanced to Associate Professor of Clinical Bacteriology in 1932 and at the same time was made Assistant Professor of Bacteriology in the Medical School of the University of Pennsylvania.

Dr. Boerner found time in his busy life to be the Bacteriologist of the Department of Health of Pennsylvania (1939–1940) as well as Serologic Field Director of that organization. He was a member of the American Veterinary Medical Association, the Pennsylvania Veterinary Medical Association, Philadelphia County Medical Society, Society of American Bacteriologists, American Public Health Association, American Society of Clinical Pathologists and Sigma Xi.

He was joint author with Dr. John Kolmer of the textbook, "Approved Laboratory Technic", a volume that has passed through several editions. At the time of his death he was working on another book as joint author to be called "Normal Values in Medicine". He contributed many papers to medical and veterinary periodicals but is best known for his work on the complement-fixation and flocculation tests which bear his name together with that of the co-author, Miss Marguerite Luckens.

Aside from his scientific accomplishments, Dr. Boerner will long be remembered by his friends and associates as a charitable, unassuming, modest gentleman. He was much beloved; all shall miss his cheerful smile, kindly word and generous help.

Louise Doddridge Larimore

Dr. Louise Doddridge Larimore died at her home in Greenwich, Connecticut, on September 20, 1948 at the age of 59 years.

She was born in New York City on March 3, 1889. Interested from her early youth in chemistry, she prepared for work in this field at Wellesley College and took her master's degree in chemistry and bacteriology at Columbia University. She studied medicine at the Woman's Medical College of Pennsylvania, graduating in 1915. She served as intern and resident physician in otolaryngology at the Woman's Hospital in Philadelphia and was appointed instructor in otology at the Woman's Medical College.

Dr. Larimore then went to New York as an assistant fellow in pathology at Rockefeller Institute. In 1923 she became research assistant in public health to Dr. Haven Emerson and from 1924 to 1928 she was instructor and associate in pathology at Columbia University. In 1926 she became pathologist at the Greenwich Hospital, beginning an association that lasted for twenty years. During her years here she acted as pathologist to the New York Infirmary for Women and Children from 1927 to 1929 and at Glen Cove Hospital from 1929 to 1939. After her retirement from the hospital she opened an office in Greenwich for the practice of allergy, in which work she continued until her death.

Dr. Larimore was a distinguished pathologist and continued to be called in consultation after her retirement from active work in this field. She was capable and skillful and was possessed of a generosity and unselfishness of spirit that made the sharing of her knowledge a pleasure to her. She will be greatly missed by her colleagues.

NEWS AND NOTICES

Letters to the Editor

February 23, 1949

To the Editor:

In the December 1948 issue of the American Journal of Clinical Pathology, Klein, Leiby and Berke[1] reported finding the optimal ratio in the Standard Kline Slide Test Antigen to be 1 part cardiolipin to 8 parts lecithin. We had previously reported[2] the optimal ratio as 1 part cardiolipin to 9.4 to 10.6 parts lecithin.

Our experience was based upon the use of Pangborn preparations with 2 mg. of cardiolipin per 1 ml. of antigen mixture. The work of Klein, Leiby and Berke is based upon the use of Lederle reagents and 2 mg. of cardiolipin per 2.6 ml. of antigen. Their antigen formula included over twice as much alcohol as the one we used and may have been responsible for the discrepancy in ratios.

Experience with LaMotte preparations to date (14 lots of lecithin and 10 lots of cardiolipin) in mixtures containing 2 mg. of cardiolipin per 1 ml. has shown the optimal ratio of these reagents to vary from 1 cardiolipin to 9 lecithin, to 1 cardiolipin to 11 lecithin.

Cleveland, Ohio B. S. Kline

References

1. Klein, S. J., Leiby, G. M., and Berke, M.: Cardiolipin antigen in the Kline test for syphilis. Am. J. Clin. Path., **18**:940–951, 1948.
2. Kline, B. S.: Cardiolipin antigen in the microscopic slide precipitation test for syphilis. Am. J. Clin. Path., **16**:68–80, 1946.

March 8, 1949

To the Editor:

Dr. Kline is correct in pointing out the variation in alcohol content of comparable cardiolipin-lecithin antigen solutions prepared from Lederle and LaMotte reagents. However, we did not feel that this variation was of a significantly large magnitude. Although our recommended cardiolipin-lecithin solution contained more than twice the amount of alcohol present in the Kline formula, the final diluted antigens show much smaller difference in alcohol content, since both antigens contain the relatively large amount of alcohol contributed by the alcoholic cholesterol solution. The diluted Kline antigen emulsion contains 1.1 ml. of alcohol per 4.4 ml. (25 per cent); our modification of the Kline antigen contained 1.26 ml. of alcohol per 4.56 ml. (27.6 per cent).

Furthermore, the factor of alcohol content was controlled in those battery tests performed with the more concentrated "experimental" lots of Lederle cardiolipin and lecithin.[1] In those experiments where parallel tests were performed with antigens of cardiolipin-lecithin ratio 1:5 to 1:10, the only variable factor was the amount of lecithin in each antigen.

It would seem, therefore, that factors other than variation in alcohol content are involved in the minor discrepancy between our results and those of Dr. Kline. We feel that further data are required to establish the optimal ratio, and have continued our battery tests with antigens having different ratios. We plan to submit our 1948 data for publication in the near future.

Van Nuys, California Sidney J. Klein

Reference

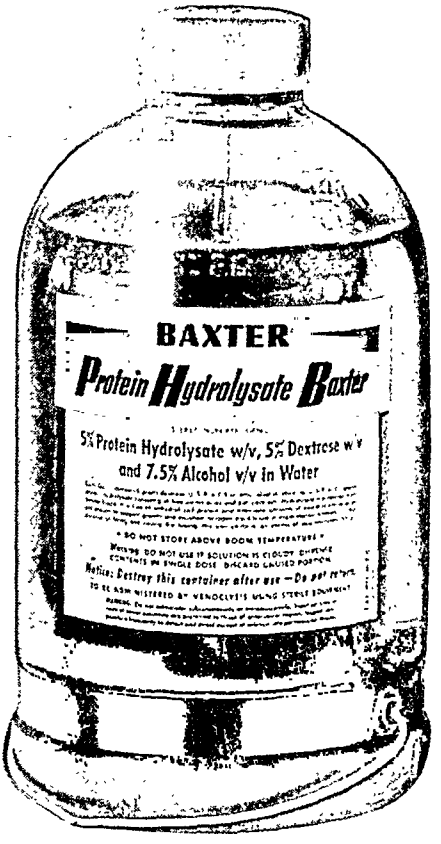
1. Klein, S. J., and Leiby, G. M.: Cardiolipin slide test in the serodiagnosis of syphilis. Am. J. Syph. Gonor. and Ven. Dis., **32**:377–390, 1948.



Announcing

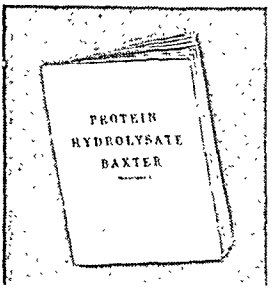
an aid to

Sedation



Many investigators have reported the value of parenteral alcohol in operative cases as a sedative and analgesic, substituting for opiates and so eliminating undesirable side effects. In introducing this solution of 5% Protein Hydrolysate w/v, 5% Dextrose w/v, and 7.5% Alcohol v/v, Baxter Laboratories offer a single solution which provides protein nourishment, caloric value and sedation.

protein and caloric requirements with minimum fluid infusion In many cases where nutrition must be provided parenterally, it has been difficult to provide sufficient calories without too much fluid. In addition to its analgesic value, this solution provides a richer available source of energy than dextrose and protein digest solutions. Each 1000 cc. provides 800 calories.



Write today for 16-page booklet giving detailed information on Protein Hydrolysate, Baxter.

product of
BAXTER Laboratories
Morton Grove, Illinois
Acton, Ontario

Distributed and available only in the 37 states east of the Rockies (except El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS

(In writing to advertisers, please mention the Journal—it helps.)

Announcement

FALL REGISTRY EXAMINATIONS

October 28, 1949

The deadline for acceptance of applications will be September 1.

Minimum requirements must have been met by the date of the examination.

Examinations will be given to candidates seeking certification as medical technologists, laboratory aides, bacteriologists, chemists, and tissue technicians.

Application blanks may be obtained from:

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
American Society of Clinical Pathologists
Muncie, Indiana

MICHAEL REESE HOSPITAL
POSTGRADUATE SCHOOL

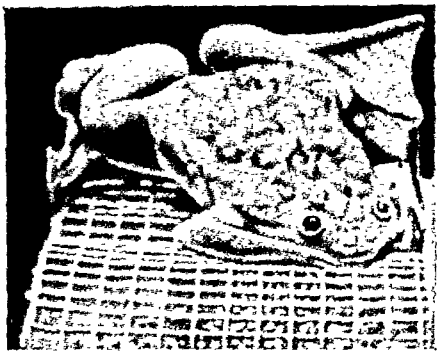
HEMATOLOGIC DIAGNOSIS

August 1–13, 1949 (Full-Time)
By
KARL SINGER, M.D.
Director of Hematologic Research

Instruction in actual reading of slides—normal and pathological specimens—Peripheral blood and bone marrow. Individual slide collection provided and may be retained by student. Review of present status of Hematology.

TUITION: $100.00

For further information, address:
Dr. Samuel Soskin, Dean
Michael Reese Hospital
Postgraduate School
29th St. & Ellis Avenue
Chicago 16, Illinois



XENOPUS LAEVIS FROG

Pregnancy Diagnosis

Animal Costs Greatly Reduced

We assure you of a continuous supply. Accurate, speedy, and inexpensive. Average animal cost per test 20 cents to 35 cents. We carry a complete stock of Laboratory Tanks, Isolation Jars, Holders, and correct reagents and apparatus for running tests. Speedy delivery via AIR EXPRESS. Current brochure showing new low prices sent upon request.

Jay E. Cook, Importer

P. O. Box 302 Baltimore 3, Md., U.S.A.
Cable Address—XENOPUS-BALTIMORE

THE PANRAY CORP.

pioneer manufacturer of DOPA

and other specialized chemicals

for investigation and research . . .

announces the availability of

Iodoacetic Acid "Panray"

(twice recrystallized, special reagent grade)

for the Iodoacetate Index Determination of Serum Proteins

Iodoacetic acid is a recently reported reagent for the determination of *deficient thermal coagulation of serum proteins* in cancer and other pathological conditions.

Originally reported by C. Huggins, M.D., G. M. Miller, M.D., and E. V. Jensen, Ph.D.: Thermal Coagulation of Serum Proteins—II. Deficient Coagulation in Cancer and the Iodoacetate Index —*Cancer Research, Vol. 9, No. 3, March 1949.*

DOPA "PANRAY"

(3,4-dihydroxyphenylalanine, C. P.)

available in l and dl forms

Synthetic chemical reagents . . . capable of revealing the presence of an oxidase in cells elaborating melanin. Valuable indicators of active melanogenesis.

Write for prices and other data

THE PANRAY CORP.

Custom Manufacturers of Fine Organic Chemicals

398 Broadway New York 13, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)

Research Opportunity

A well trained research worker is required to conduct experiments in the biological aspects of 25 mev X-rays and electrons. A Betatron at the University of Saskatchewan is available. Applicants should be capable of independent research and, therefore, must have had previous experience in radio-biological research. The appointment is for a minimum of two years. Salary by arrangement. Further information may be obtained from: Dr. T. A. Watson, Director, Cancer Clinic, City Hospital, Saskatoon, Saskatchewan, Canada.

TRYPTICASE TELLURITE AGAR

Maryland Formula

A highly selective plating medium which supports good growth of *intermedius* as well as other types of *C. diphtheriae* and is recommended for the laboratory diagnosis of diphtheria.

Write for new B-B-L Catalog
"Products for the Bacteriological Laboratory"

Literature and prices are sent on request

BALTIMORE BIOLOGICAL LABORATORY

1640 Gorsuch Avenue Baltimore-18, Md.

FOR BEST RESULTS USE

USSLA Products

Krautman's:

STABLE BUFFERED STARCH AND SPECIAL DEPROTEINIZING REAGENT are ready at all times for the *immediate, accurate,* and *simple* analysis of BLOOD AMYLASE. Reagents for 65 determinations $4.75

KRAUTMAN'S DEPROTEINIZING REAGENT SALT is used to make a stable reagent for the *preparation of protein-free blood filtrates* for such determinations as Urea N, Glucose, Uric Acid, and Creatinin,
One bottle for 2 liters of solution $2.50
Five btls., each for 2 l. of solution $9.35

NESSLER'S 'IODIZED' SALT is *ideal* for the preparation of Working Nessler's Solution. This solution is *GUARANTEED* to give *accurate Results* in the determination of Urea N in Blood and Urine.
68 gms. for 1 lit. of sol..... $1.90

U. S. Scientific Laboratories

237 Christopher Ave. Brooklyn 12, N. Y.

ALBIMI

PREPARED CULTURE MEDIA

In screw-capped one ounce bottles providing a large surface area

LOEFFLER'S COAGULATED BLOOD SERUM

LÖWENSTEIN'S MEDIUM
For tubercle bacilli

PETRAGNANI'S MEDIUM
For tubercle bacilli

Other prepared media available
Literature on request

ALBIMI LABORATORIES, Inc.

16 CLINTON STREET
BROOKLYN 2, N. Y.

for

BLOOD TYPING
RH TESTING

you need the highest-titred serum available.

ANTI-HUMAN PRECIPITIN SERUM (rabbit)
for the Coombs Test

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human Red Cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic Serum
for control tests

P.S.A.
(Paraffin Section Adhesive)

Write for complete information.

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York

"VACSEAL"

MEANS DEPENDABILITY IN GUINEA PIG COMPLEMENT

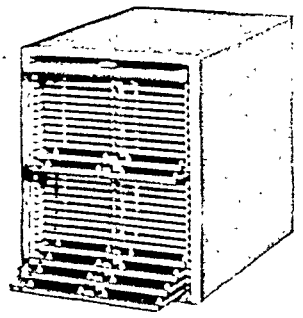
Since 1939, Carworth Farms has been the largest producer of guinea pig complement in this country. We are now distributing this material under our own label, "Vacseal", at a more favorable price.

It is well known that the production of this biologic requires great care. Our long experience and large output enable us to offer a superior product to the laboratory worker. We invite you to try "Vacseal" Guinea Pig Complement, available in glass ampules with a special diluent for restoration to the following amounts:

3 cc 7 cc 20 cc

Any recognized laboratory will receive a trial 3 cc package on request.

CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK

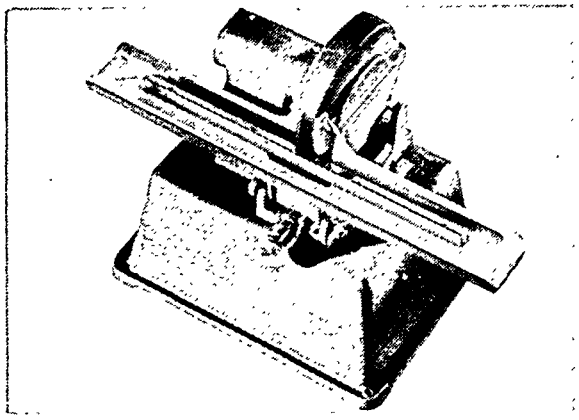


EBERBACH UNIT SYSTEM MICRO SLIDE CABINETS

You can have an "expandable" filing system with the slides easy to locate. Each cabinet holds 500 slides flat in individual compartments. Hardwood cabinets 8½ x 10 x 12" deep have flat slides and disappearing door so as to stack neatly. # 60-660 cabinet shown above sells for $36.00. Write for Bulletin 150.

Eberbach & Son Company
LABORATORY APPARATUS & SUPPLIES
ANN ARBOR, MICH. ESTABLISHED 1849

INDISPENSABLE
in your laboratory...



the Berkeley
MICROTOME KNIFE SHARPENER
for perfect edges...three times faster

Operation is simple, eliminates honing and stropping. Assures hollow-ground edges that cut better and last much longer. *Send for our Illustrated Bulletin MKS-14*

Berkeley Scientific Company
SIXTH AND NEVIN AVENUE · RICHMOND, CALIF.

SHILLABER'S
Non-Drying
IMMERSION OIL
for Microscopy

OFFERS THESE ADVANTAGES OVER CEDAR OIL..

- *Better Results
- *Safer
- *Time Saving
- *Neutral
- *Low Fluorescence
- *2 Viscosity Ranges
- *Controlled Optical Properties



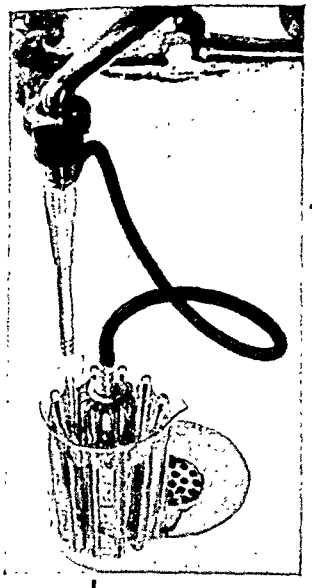
PRICES (Grade A or Grade B)

COMBINATION PACKET	
1 Ounce A with 1 Ounce B	$1.00
1 Ounce Bottle Each	.50
4 Ounce Stock Bottle	1.50
16 Ounce Stock Bottle	4.00

ONE OUNCE BOTTLES HAVE APPLICATOR RODS

Order Through Your Laboratory Supply Dealer Or Direct From

R. P. Cargille 118 Liberty Street New York 6, N. Y.



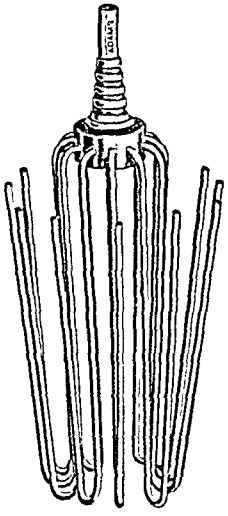
for rapid, effective cleaning of **WINTROBE HEMATOCRIT TUBES**

Clean your Wintrobe hematocrit tubes more quickly and more easily with Adams stainless steel, suction type automatic cleaners . . . Eliminate breakage, assure thorough cleaning.

12-Unit Cleaner

Connect suction outlet of cleaner to a standard water suction apparatus with a rubber hose. Place inverted hematocrit tubes over cleaner U-tubes, set cleaner in a beaker and run water into beaker, providing a constant water supply. When suction is turned on, blood is drawn from hematocrit and water follows, flushing the tubes. When cleaning fewer than 12 hematocrits, stopper unused U-tubes to prevent loss of suction.

A-2467 12-unit cleaner, with 12 rubber stoppers; without suction apparatus.....**each $15.00**

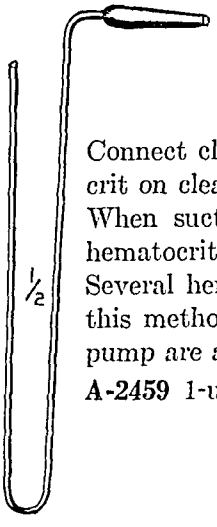


A-2467

1-Unit Cleaner

Connect cleaner to a suction pump, invert hematocrit on cleaner U-tube and set in a beaker of water. When suction is turned on, blood is drawn from hematocrit and water follows, flushing the tube. Several hematocrits can be cleaned at one time by this method if suitable attachments to the suction pump are available.

A-2459 1-unit cleaner.....**each $ 2.50**
doz. 25.20



A-2459

Write for Circular 448CP describing our full line of Blood Sedimentation Apparatus

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

ADAMS

Coming Soon!

Handbook of

DIGESTIVE DISEASES

by JOHN L. KANTOR, M.D., F.A.C.P., Late Associate in Medicine, Columbia University; Gastroenterologist and Associate Roentgenologist, Montefiore Hospital for Chronic Diseases, New York; and ANTHONY M. L. KASICH, M.D., Adjunct Physician, Montefiore, Hospital; Assistant Visiting Physician, Bellevue Hospital, New York.

(In Preparation)

This is a new and enlarged edition of Dr. Kantor's Synopsis of DIGESTIVE DISEASES. Work on the revision was interrupted by Dr. Kantor's death, and the task was taken up by Dr. Kasich.

Because of the recent accumulation of new and important information resulting from research in gastroenterology, Dr. Kasich expanded the book, thus taking it out of the Synopsis Series.

In bringing it completely up to date, it has been entirely rewritten. It includes all essential and salient facts concerning gastrointestinal diseases. Dr. Kasich has written with a twofold purpose: first, to present a concise picture of the more vital and fundamental aspects of digestive diseases; second, to approach the subject on a sound physiological basis. The fact that gastroenterology is an important and inseparable segment of internal medicine has been stressed throughout.

CONTENTS

Classification of Digestive Diseases.
Diagnostic Methods; History and Physical Examination, Special Tests.
Organic Constitutional Inferiority: The Anomalies.
Diseases of the Mouth.
Diseases of the Esophagus.
Diseases of the Stomach (Four Chapters)
Diseases of the Small Intestine (Two Chapters).
Diseases of the Colon (Two Chapters).
Diseases of the Appendix.
Diseases of the Rectum and Anus.
Diseases of the Liver (Three Chapters).
Diseases of the Gallbladder and Bile Ducts.
Diseases of the Pancreas.
Intestinal Parasites:
The Protozoa
The Flatworms: Trematodes
The Roundworms.
Digestive Symptoms in Extradigestive Diseases.
Psychiatric Aspects of Digestive Diseases.
Gastrointestinal Allergy.

ORDER FORM

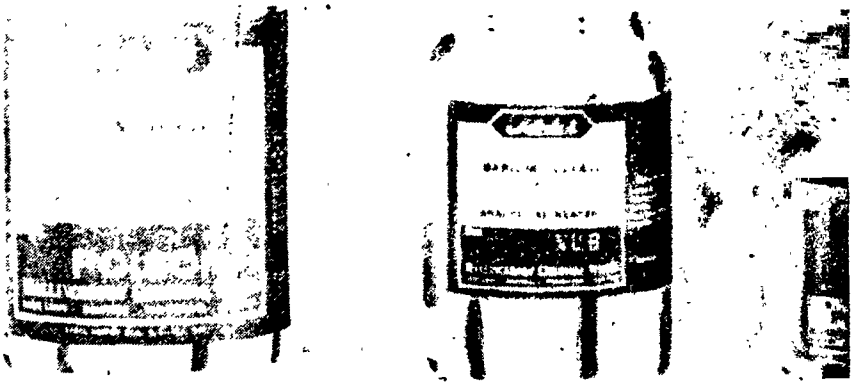
The C. V. Mosby Company
3257 Washington Blvd.
St. Louis 3, Missouri

AJCP 6-49

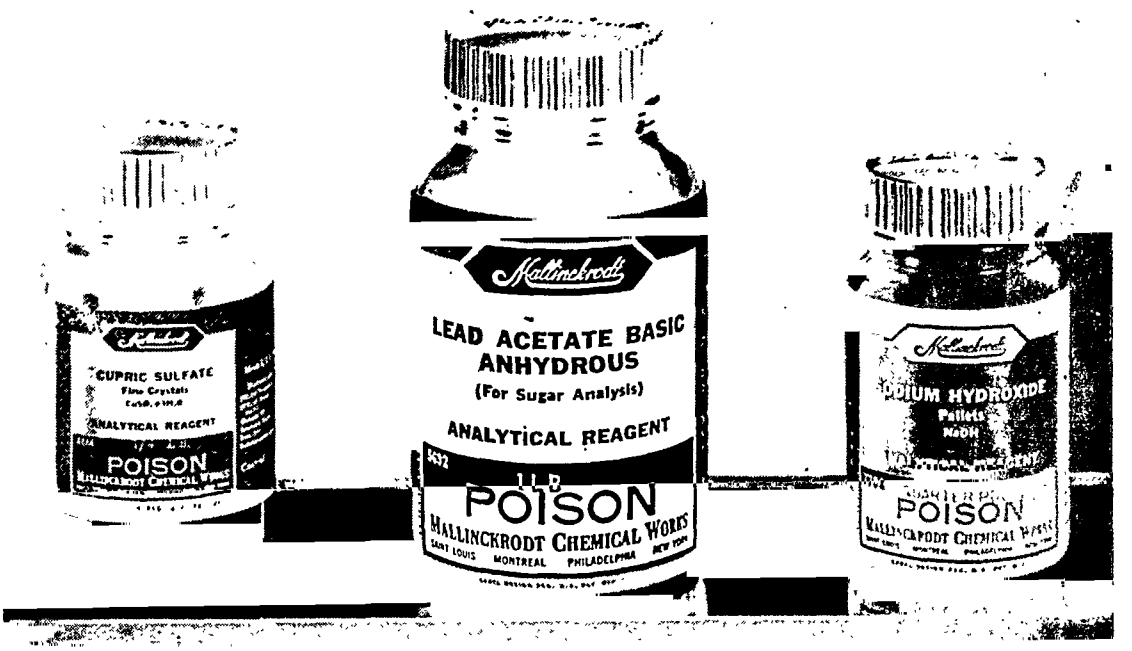
Please send me a copy of Kantor-Kasich HANDBOOK OF DIGESTIVE DISEASES when ready.
☐ Bill me when shipped. ☐ Send C.O.D.

Name

Address



for Sugar Analyses



82 Years of Service to Chemical Users

MALLINCKRODT CHEMICAL WORKS

MALLINCKRODT STREET, ST. LOUIS 7, MO. • 72 GOLD STREET, NEW YORK 8, N. Y.

CHICAGO • CINCINNATI • CLEVELAND • LOS ANGELES • MONTREAL • PHILADELPHIA • SAN FRANCISCO

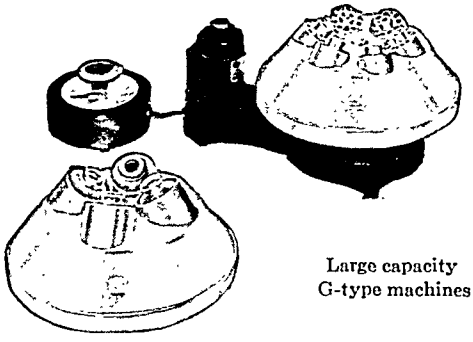
UNIFORM DEPENDABLE PURITY

(In writing to advertisers, please mention the Journal—it helps.)

SERVALL

ANGLE CENTRIFUGES

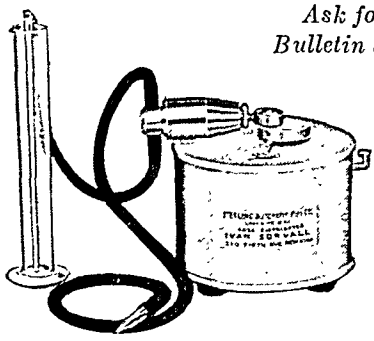
Ask for Bulletin J-61
with complete data on all models



Large capacity
G-type machines

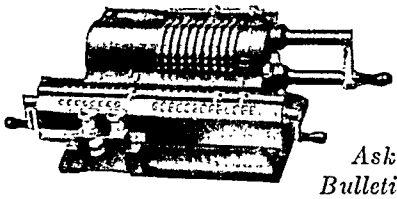
Type G/1, for 6 bottles of 250 cc each
18 tubes of 60 cc each
42 tubes of 15 cc each
78 tubes of 10 cc each
Type G/2, for 4 bottles of 500 cc each
Type G/3, for 10 tubes of 100 cc each

Ask for Bulletin J-62



STERLING AUTOMATIC AND HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.
Easy to operate—Time-Saving



Ask for Bulletin J-63

ORIGINAL—ODHNER

Portable Calculator for the Scientist
Many different models
Efficient - Sturdy - Low Priced

Manufacturers and Distributors:

IVAN SORVALL, Inc.
210 FIFTH AVE.-NEW YORK 10, N. Y.

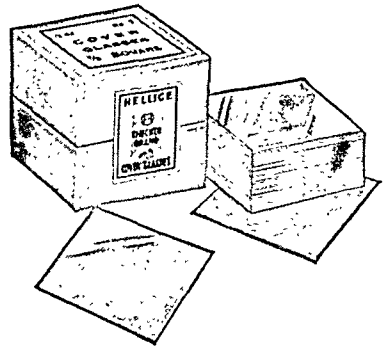
HELLIGE

CHECKER BRAND

MICRO

COVER GLASSES

Again Available!



We have resumed the production of micro cover glasses, discontinued during the war because our efforts were directed primarily toward manufacturing for the U.S. Army and Navy Hellige specialties not obtainable elsewhere.

Now we are able to offer again the same surpassing quality which made Hellige Micro Cover Glasses the choice of the medical profession for more than half a century.

Write Today for our Attractive Prices

HELLIGE
INCORPORATED
3718 NORTHERN BLVD.
LONG ISLAND CITY 1, N.Y.

AT HOME OR AWAY SPOT TESTS SIMPLIFY URINALYSIS

No Test Tubes • No Measuring • No Boiling

Diabetics welcome "Spot Tests", (ready to use dry reagents), because of the ease and simplicity in using. No test tubes, no boiling, no measuring; just a little powder, a little urine—color reaction occurs at once if sugar or acetone is present.

Galatest . . . Acetone Test (DENCO)

FOR DETECTION OF SUGAR IN THE URINE — FOR DETECTION OF ACETONE IN THE URINE

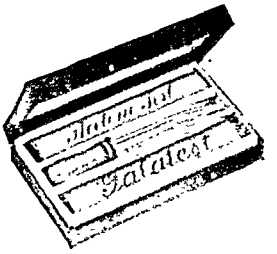
SAME SIMPLE TECHNIQUE FOR BOTH

1. A LITTLE POWDER — 2. A LITTLE URINE

COLOR REACTION IMMEDIATELY

Accepted for advertising in the Journal of the A.M.A.

WRITE FOR DESCRIPTIVE LITERATURE



A carrying case containing one vial of Acetone Test (Denco), one vial of Galatest, medicine dropper and Galatest color chart is now available at all prescription pharmacies and surgical supply houses. This is very convenient for the medical bag or for the diabetic patient.

Acetone Test (DENCO) *Galatest* — THE DENVER CHEMICAL MANUFACTURING COMPANY, INC. 163 Varick St., New York 13, N. Y.

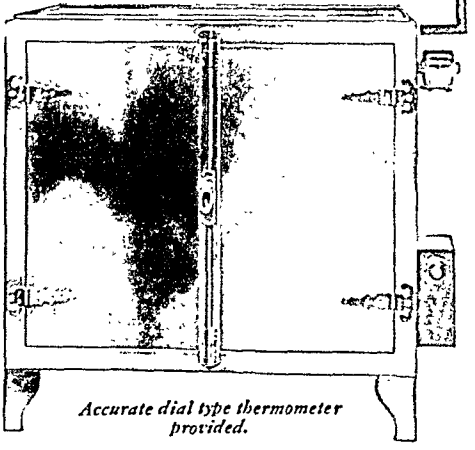
Castle **Hot Air Sterilizer**

LIFETIME STAINLESS STEEL . . . FOR CONSTANT, EXTRA HEAVY WORK IN THE LABORATORY

The Castle No. 3 Hot Air Sterilizer, by reason of its insulated *and* stainless steel construction, gives longer service, and more accuracy of temperature control.

Circulating fan, powered by heat-resisting motor on outside wall, provides full circulation of air . . . eliminates "hot" and "cold" spots . . . insures uniformity of 4°C.

Guaranteed electrical heating units inside sterilizer, with automatic control, hold temperature at *any* point from 38° to 260°C. Exceptional accuracy of control enables Sterilizer to be used also as a Drying Oven.



Accurate dial type thermometer provided.

For full details, write: Wilmot Castle Company, 1216 University Ave., Rochester 7, N. Y.

Castle **BACTERIOLOGICAL APPARATUS**

NEW UP-TO-DATE

ORAL VACCINES

& IMMUNIZATION BY OTHER UNUSUAL ROUTES

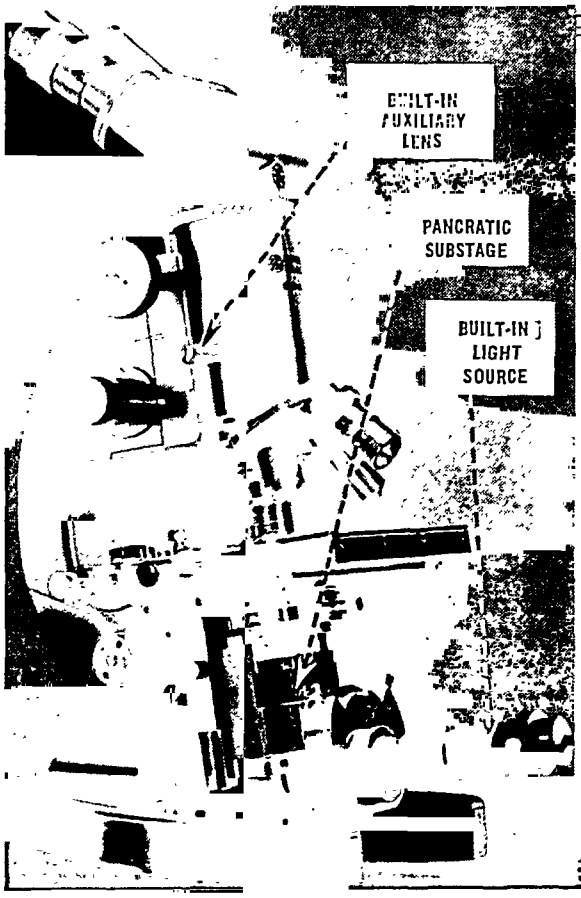
By DAVID THOMSON, M.D.
Director, Pickett-Thomson Research Laboratories

and ROBERT THOMSON, M.B.
Pathologist, St. Paul's Hospital, London

Assisted by
James Todd Morrison, M.D., D.P.H.,
Aberdeen, Scotland

Here organized in one volume are the results of world research on oral vaccines and other immunizations by non-parenteral routes. Over 1000 original research papers have been analyzed and summarized in chronological order. Of especial interest to pediatricians and practitioners are the indications of greater tolerance shown in children. *342 pp., 5 figs. $11.00*

THE WILLIAMS & WILKINS COMPANY
Mt. Royal & Guilford Aves. Baltimore 2, Md.



GALILEO CORP. OF AMERICA, Inc.

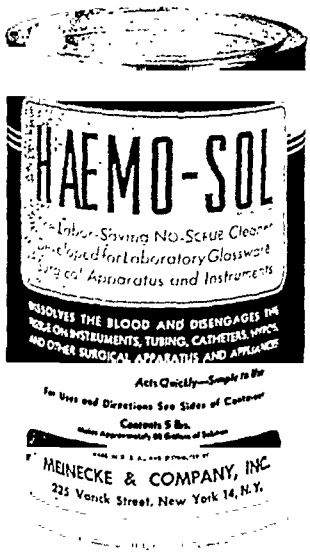
Announces

The VNP Phase-Contrast Microscope with features which open completely new possibilities in phase-contrast technique.

•

For descriptive brochure write
PFALTZ & BAUER, Inc.
Empire State Building, New York 1, N. Y.
Sole agents for
GALILEO CORPORATION OF AMERICA, Inc.
745 Fifth Avenue, New York 22, N. Y.
Distributor for Officine Galileo, Italy
Florence • Milan • Venice
Since 1881 manufacturers of research microscopes and microscopical equipment of all kinds, refractometers, polarimeters, and analytical balances.

(In writing to advertisers, please mention the Journal—it helps.)



Literature and samples on request. Write us regarding your specific cleaning problems.

NO RESIDUE

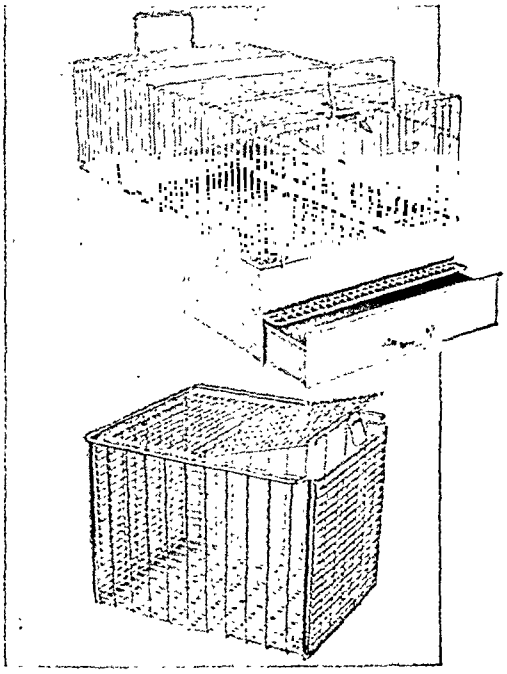
TO INTERFERE WITH YOUR EXACTING TITRATIONS AND TESTS

Good technique demands that laboratory glassware be not merely washed but *thoroughly cleansed.* This you can be sure of when you use HAEMO-SOL, the original chemically prepared cleaning agent and blood solvent, specifically developed for laboratory and hospital work.

Of equal importance, laboratory glassware must be free of even the slightest trace of detergent-induced deposit or residue. HAEMO-SOL affords you this protection due to its extraordinary solubility and free rinsing qualities, indicated by the fact that it dissolves to a crystal-clear solution evincing no turbidity or precipitate on standing. Furthermore, HAEMO-SOL'S non-etching effect on glass, correlated with its low pH, saves costly replacement of laboratory apparatus.

MEINECKE & COMPANY, INC.
225 VARICK STREET • NEW YORK 14, N. Y.

FOR THE MOST DISCRIMINATING TECHNICIAN



Laboratory Animal Cages by *Bussey*



Bussey cages are the ultimate in cleanliness and ease of servicing. Several specific-purpose cages, one all-purpose cage...for guinea pigs, hamsters, rat colonies, small rabbits and cats. Racks designed for each type of cage. Cages are suspended, with dropping pans as separate units. Finest electric welded construction. Finish specially resistant to strong cleansing and disinfectant solutions. Write for literature and prices. Free consultation on special equipment problems. Write today.

Prompt Delivery

BUSSEY PRODUCTS CO.
6000-55 W. 51st St. • Chicago 38, Ill.

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia...O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Benbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

American Society of Clinical Pathologists

OFFICERS

President: Dr. O. A. Brines, Wayne University College of Medicine, Detroit 26, Michigan
Vice-President: Dr. C. H. Manlove, 5725 S.E. Stark Street, Portland 15, Oregon
President-Elect: Dr. J. B. McNaught, University of Colorado School of Medicine, Denver, Colorado
Secretary-Treasurer: Dr. C. G. Culbertson, Indiana University Medical Center, 1040-1232 West Michigan Street, Indianapolis 7, Indiana

STANDING COMMITTEES

EXECUTIVE COMMITTEE

W. S. Thomas, Chairman, Rochester, N. Y.
T. J. Curphey, Long Island, N. Y.
F. W. Konzelmann, Atlantic City, N. J.
L. W. Larson, Bismarck, N. D.
S. P. Reimann, Philadelphia, Pa.
J. R. Schenken, Omaha, Neb.

BOARD OF CENSORS

L. W. Diggs, Chairman, Memphis, Tenn.
J. J. Andujar, Fort Worth, Tex.
F. C. Coleman, Des Moines, Iowa
J. W. Howard, Wilmington, Del.
V. M. Johnson, West Palm Beach, Fla.
C. P. Larson, Tacoma, Wash.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. Montgomery, Chairman, Muncie, Ind.
A. G. Foord, Pasadena, Cal.
F. B. Lynch, Jr., Philadelphia, Pa.
F. B. Queen, Portland, Ore.
R. G. Stillman, New York, N. Y.
A. H. Wells, Duluth, Minn.

APPOINTED COMMITTEES 1948-1949

GENERAL RESEARCH COMMITTEE

M. Strumia, Chairman
B. S. Kline
A. H. Sanford
W. M. Simpson

COMMITTEE ON HEMATOLOGIC TERMINOLOGY

E. E. Osgood, Chairman
L. Berman
R. R. Kracke
F. J. Heck
L. R. Limarzi

BLOOD BANK COMMITTEE

D. H. Kaump, Chairman
L. Y. Dyrenforth
L. W. Larson
F. W. Hartman
R. G. Stillman

COMMITTEE ON REGISTRY OF GENERAL TUMORS

J. W. Kernohan, Chairman
L. V. Ackerman
Col. J. E. Ash
W. A. D. Anderson
S. W. Lippincott
I. Perry

REPRESENTATIVE ON NATIONAL RESEARCH COUNCIL SUBCOMMITTEE ON ONCOLOGY

S. Warren

REPRESENTATIVE ON BOARD OF GOVERNORS OF COLLEGE OF AMERICAN PATHOLOGISTS

T. J. Curphey

COMMITTEE ON SEMINAR

I. Davidsohn, Chairman
P. R. Cannon
J. B. McNaught
Gen. R. O. Dart
R. A. Moore

PROGRAM COMMITTEE

C. G. Culbertson, Chairman
R. A. Moore, Co-Chairman
C. J. Bucher
F. B. Queen
F. W. Sunderman

COMMITTEE ON NECROLOGY

H. R. Brown

COMMITTEE ON EDUCATION AND PUBLICITY

W. O. Russell, Chairman
J. L. Carr
W. M. Germain
E. F. Hirsch
D. A. Nickerson
S. P. Reimann

COMMITTEE TO STANDARDIZE EDUCATION AND TRAINING OF MEDICAL TECHNOLOGISTS

J. J. Andujar, Chairman
F. C. Coleman
E. Moss
O. O. Christianson
M. Oosting
R. Miller
P. Rosenblatt
J. P. Tollman

DIFCO

DETECTION OF THE GONOCOCCUS

The superiority of the cultural method over the usual microscopic technique for detection of gonococcal infections has repeatedly been demonstrated. The cultural method is especially indicated in chronic and treated cases and for determining the release of patients. The use of the following standardized materials has simplified the diagnosis of gonorrhoea by the cultural method.

BACTO-G C MEDIUM BASE

enriched with Bacto-Hemoglobin and Bacto-Supplement A or Bacto-Supplement B is the most suitable medium available for cultural detection of *Neisseria gonorrhoeae* from all types of gonococcal infections. This new medium requires only 24 hours incubation. Upon this medium gonococcus colonies may be detected even when the organisms are present in the exudate in such small numbers that they may be missed upon microscopic examination.

BACTO-PROTEOSE NO. 3 AGAR

enriched with Bacto-Hemoglobin, is the original medium developed by Difco for isolation of the gonococcus and has been widely used for many years.

BACTO-SUPPLEMENT A
BACTO-SUPPLEMENT B

were developed as enrichments for chocolate agar prepared from Bacto-G C Medium Base or Bacto-Proteose No. 3 Agar with Bacto-Hemoglobin to furnish the essential growth factors required by some strains of *Neisseria gonorrhoeae.*

PROTEOSE PEPTONE NO. 3

is the basic ingredient of Bacto-G C Medium Base and Bacto-Proteose No. 3 Agar. This peptone, in a saline solution, is also recommended as a diluent for suspending the exudate prior to inoculation on chocolate agar.

BACTO-HEMOGLOBIN

in a two per cent solution is the preferred enrichment for preparation of chocolate agar from Bacto-G C Medium Base or Bacto-Proteose No. 3 Agar. It is readily soluble in water and is sterilized in the autoclave.

BACTO-DEXTROSE STARCH AGAR

is a very useful medium for propagation of pure cultures of the gonococcus. The organism grows luxuriantly upon this medium without further enrichment. Bacto-Dextrose Starch Agar also supports excellent growth of many other pathogenic bacteria and when used in half strength it is well suited for maintaining stock cultures.

BACTO-PHENOL RED MEDIA

are particularly useful for determination of fermentative reactions of newly isolated strains of the gonococcus. A selected group of complete agar and broth media are available, as are also the basic media without carbohydrate.

Leaflet No. 121 "Diagnosis of Gonococcal Infections by the Cultural Method" available on request

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES

DETROIT 1, MICHIGAN

American Journal of Clinical Pathology

VOLUME 19 APRIL 1949 NUMBER 4

CONTENTS

Symposium on the Cytologic Diagnosis of Cancer

Cytologic Diagnosis of Uterine Cancer by Examination of Vaginal and Uterine Secretions. G. N. PAPANICOLAOU 301

Cytologic Diagnosis of Cancer from Pleural and Peritoneal Fluids. O. SAPHIR 309

Cytologic Diagnosis of Carcinoma of the Prostate. P. A. HERBUT 315

Tumor Cells in Bronchial Secretions. An Experimental and Statistical Study. M. APPEL AND T. T. BRONK 320

A Cytohistologic Study of Bronchial Secretions. H. L. RICHARDSON, W. C. HUNTER, W. S. CONKLIN AND A. B. PETERSEN 323

Cytohistologic Diagnosis of Material Aspirated from Stomach. Accuracy of Diagnosis of Cancer, Ulcer and Gastritis from Paraffin-Embedded Washings. H. L. RICHARDSON, F. B. QUEEN AND F. H. BISHOP 328

Discussion of Papers. W. BOYD 341

General Discussion. P. A. HERBUT, M. G. BOHROD, W. FREEMAN, S. M. RABSON AND G. N. PAPANICOLAOU 343

Universal Serologic Reaction with Lipid Antigen.

I. In Normal Persons. R. L. KAHN 347

II. In Animals. R. L. KAHN AND B. J. BARIBEAU . . 361

The Pathologist and Continuation of Medical Education in a Private Hospital. A. H. WELLS 369

The Clinical Effect of Nitrogen Mustard on Hodgkin's Disease. L. A. ERF AND R. D. BAUER 372

Effect of Caronamide on Excretion of Phenosulfonphthalein. W. P. BOGER AND J. W. CROSSON 381

Clinicopathologic Conference. C. H. BINFORD AND J. B. SPRIGGS . . 388

Editorial: "Round Cell Infiltration". S. M. RABSON 393

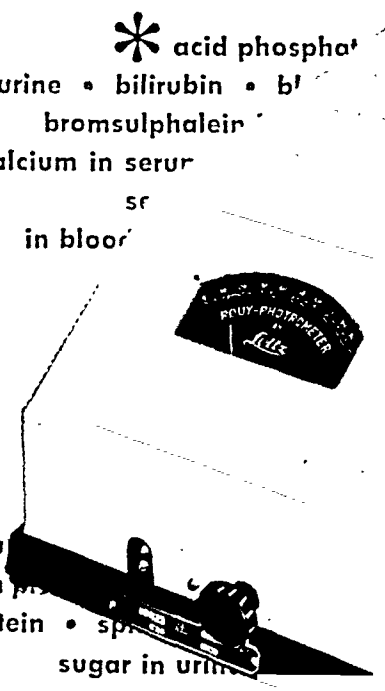
Selected Abstracts 394

Book Reviews 395

News and Notices 399

Pre-calibrated for 38 clinical tests *

* acid phospha[tase] ... albumin in
urine • bilirubin • b[lood] ... • blood thiocyanates
bromsulphalein ... test
calcium in serum ... rol in
s[erum] ... erol esters • creatinine
in blood ... in blood
... e-micro method
... bin
... x • non-protein nitrogen
... nephthalein kidney
... O_2 capacity
... nides • serum chloride
... oulin
seru[m] ... ate • serum alkaline phosphatase
serum p[rotein] ... n test
protein • sp[ecific gravity] ... albumin
sugar in urin[e]
sulfadiazine • ...
sulfaguanidine
sulfanilar[amide]
sulfapyridine • ...
sulfathiazole • urea nitr[ogen]
urea nitrogen
uric acid in blood • urine c[hlorides]



the ROUY PHOTROMETER by Leitz

Pre-calibration of the Photrometer enables you to run 38 of the common clinical tests without preparing standards... without calculation. Accurately prepared reagents are quickly available through the Leitz Solution Supply Service.

An Individual Handbook accompanies each Photrometer and contains individually prepared calibration tables that eliminate any need for calculation. In addition, the Handbook lists standard clinical methods which any competent laboratory worker can easily follow.

Ideally simple, the Photrometer has only two moving parts, requires no special skill of the user. Its convenience of operation is combined with the precision and sturdiness associated with all products bearing the name E. Leitz, Inc. Prolonged service without repairs or adjustments can be expected.

For price and details, write

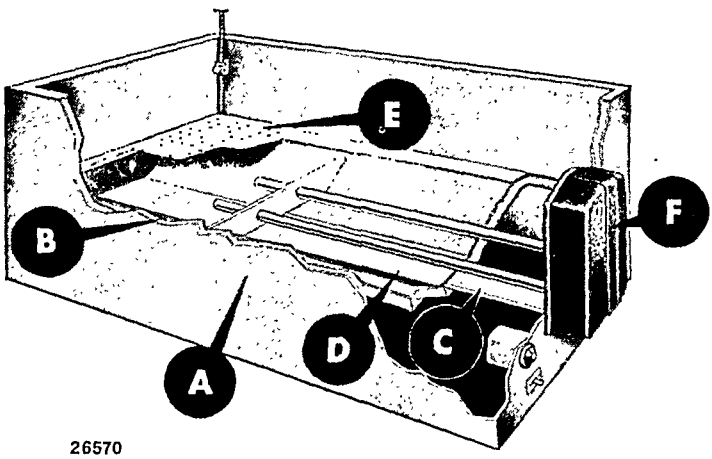
E. LEITZ, Inc., 304 Hudson Street, New York 13, N. Y.

LEITZ SCIENTIFIC INSTRUMENTS • MICROSCOPES • BINOCULARS • LEICA CAMERAS AND ACCESSORIES

STAINLESS STEEL SEROLOGICAL WATER BATH

For Constant Temperature with Precise Control

Within Plus or Minus ¼ Degree Centigrade in Any Part of Bath



26570

Meets All Demands of the Critical Laboratory Technician

This high quality stainless steel bath features easy changeover from 37.5°C. use to 56°C. use by a quick flick of the 3-position switch. It can also be reset to any specific temperature within its range from room temperature to 65°C. by adjusting knobs on thermoregulator. Indicator light aids this adjusting.

Temperatures are accurately controlled by twin thermoregulators each having metal rod immersion elements extending along the length of bath directly above the strip heater. Temperature changes cause elongations and contractions of the rods which are multipled by a lever device making the regulation extremely critical but trouble-free.

The bath will accommodate standard Kahn, Wassermann, Kolmer and Army Medical racks.

IMPORTANT FEATURES

A. Streamlined 18-8 stainless steel double wall construction throughout. Non-corrosive and easy to keep clean. Beautiful satin finish exterior.
B. Insulation of ½-inch "Fiberglass" between double walls surrounds entire water chamber and minimizes heat loss.
C. Low wattage strip element extends entire length of chamber bottom. Responds quickly to temperature control.
D. V-shaped bath chamber facilitates flow of convection currents to insure even temperature throughout bath.
E. Removable stainless steel tray has holes spaced 1" apart to allow free circulation of convection currents.
F. All control mechanisms located on end of bath are encased in dust-proof housing—prevents jarring adjustments.

26570—WATER BATH, Serological, Double Temperature Model. Of stainless steel double wall construction, with settings for either 37.5°C. or 56°C. Range from room temperature to 65°C. Complete with corp, plug and thermometer, but not racks or cover. For 115 volt A.C.

	Size 1	Size 2	Size 3
Width, length and depth (above tray) in inches	6½x12x5	12½x18x5	12½x24x5
Each	84.50	114.75	128.50

Stainless steel single temperature baths, single wall nickel plated copper baths and baths for 220 volt operation are available on request.

Write, Teletypewrite, Wire or Phone Dept. JCP-4, Our Nearest Office or Warehouse

SERVING SCIENCE THROUGH SUPPLY

WILL CORPORATION
LABORATORY APPARATUS AND CHEMICALS
ROCHESTER 3, N. Y. • NEW YORK 12, N. Y.
SOUTHERN SCIENTIFIC CO., INC., ATLANTA 3, GA.
BUFFALO APPARATUS CORP., BUFFALO 5, N. Y.

TESTING REAGENTS
for
Hematology and Serology

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
or determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Coombs' Serum
for antibody test

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human red cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic serum
for control tests

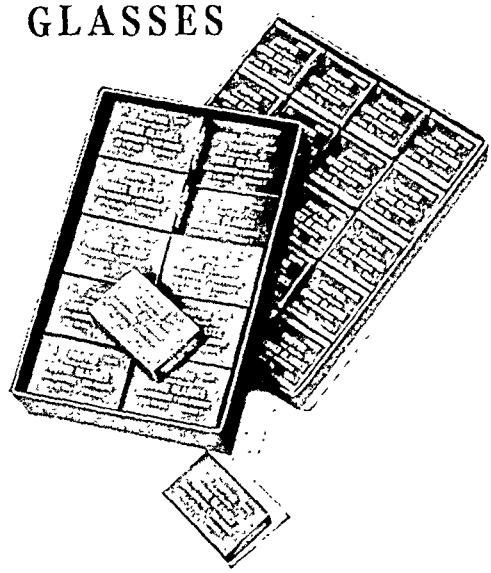
P.S.A. (Paraffin Section Adhesive)

CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York

Know Your Product

MICROSCOPIC COVER GLASSES



ESSENTIAL QUALITIES

1. **Thinness.**
2. **Uniformity of thinness.**
3. **Non-corrosive and non-fogging resistant glass.**
4. **Correctly cut edges.**
5. **Proper packaging.**

WHY ARE THESE QUALITIES NECESSARY?

Thinness: Only the precisioned thin cover glass can perform the mounting functions perfectly.

Uniformity of thinness: Assures a plane cover glass free of distortive qualities.

Non-corrosive and non-fogging glass: Permits inspection of specimen at any time after mounting. The glass will always remain clear, clean and durable.

Quality of cut edges: Prevents injury during use. Permits handling along edges without danger of cuts.

Proper packaging: Handling and constant use dictate a cellophane wrapped cover glass, protected by cotton padding and supported by a sturdy reinforced container.

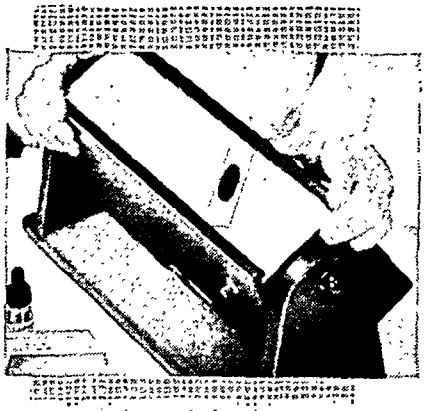
Propper Mfg. Co., Inc. L. I. C., N. Y.

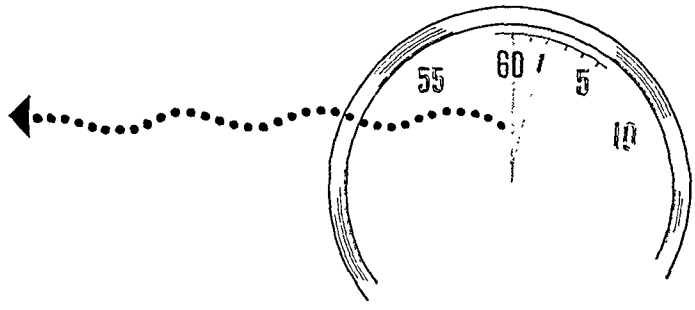
The Propper name is your guarantee of greater value.

TWO drops of blood

ONE drop of anti-Rh serum

. . . Test complete in TWO minutes or less

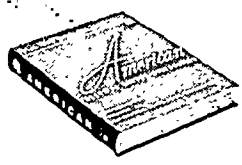




Rapid, accurate, satisfying results in blood testing are a certainty with **Rh** serums from AMERICAN. No microscope is needed. The Slide Method gives clear, distinct, unmistakable readings to the naked eye. Clumping starts in 45 seconds—is complete in from 1 to 2 minutes.

These potent and stable serums may be used with fresh, clotted, oxalated, heparinized or citrated blood. Serums are prepared from immunized *human* donors in a Government licensed laboratory—in strict accord with NATIONAL INSTITUTE OF HEALTH specifications.

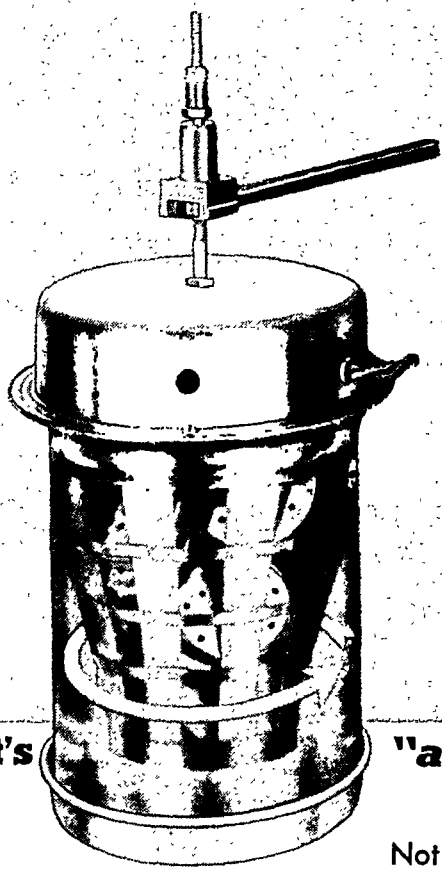
WRITE TODAY *for descriptive literature on* AMERICAN'S *complete line of blood-typing and blood-grouping serums.*



PLAN WITH AMERICAN
. . . the first name in hospital supplies

AMERICAN HOSPITAL SUPPLY CORPORATION
GENERAL OFFICES • EVANSTON, ILLINOIS

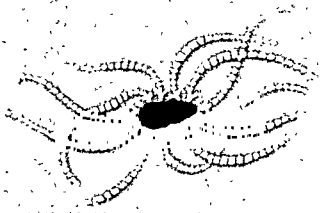
(In writing to advertisers, please mention the Journal—it helps.)





"HAND" PROCESSING

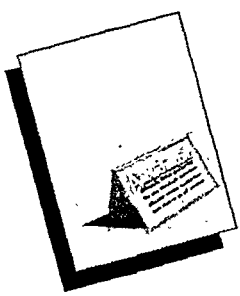
The tissue, lying at rest merely steeping in a reagent, is surrounded by a "skin" of relatively inactive solution. Permeation is slow, uncertain.



"AUTOTECHNICON" PROCESSING

The tissue moves about in the fluid, as it revolves in the carrier basket. Fresh surfaces are constantly being presented for penetration by a reagent which is, itself, in a state of gentle agitation through the constant rotation of the basket.

it's *"active displacement"* that does it



A brochure describing the Autotechnicon is available on request.

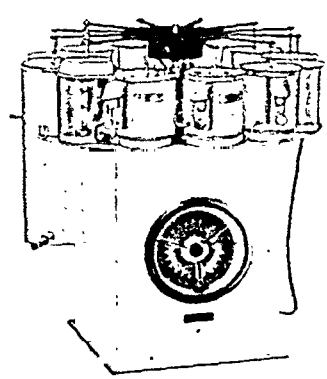
Not only does the **Autotechnicon** relieve the laboratory staff of the tedious task of processing tissues "by hand," but it also completes the chosen technique *faster, more thoroughly, more uniformly, more dependably.* The **Autotechnicon** is versatile . . . it fixes, dehydrates, washes, stains . . . delivers beautifully finished tissues ready for diagnosis.

And because it needs no human intervention, it knows no "working day." To-day's tissues, processed overnight by your own technique, will be waiting for you in the morning.

the Autotechnicon

Trade Mark Registered U. S. Patent Off.

automatic tissue processing by any histologic technique

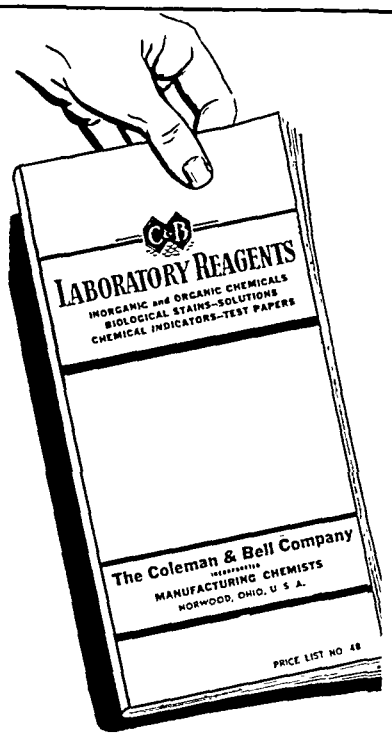


THE TECHNICON COMPANY

215 EAST 149th STREET • NEW YORK 51, N.Y.

C&B

LABORATORY REAGENTS



New Catalog FREE

If you have not received a copy of this new catalog please notify us at once.

Inorganic and Organic Chemicals

•

Biological Stains Solutions

•

Chemical Indicators Test Papers

•

Distributed by
Physician and Laboratory Supply Houses

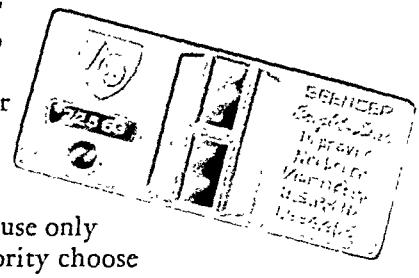
The COLEMAN & BELL Co., Inc.

Manufacturing Chemists : Norwood, Ohio, U. S. A.

COLEMAN & BELL

(In writing to advertisers, please mention the Journal—it helps.)

Only an ACCURATE Blood Count is Worth Taking



To aid in correct diagnosis and proper treatment of patients, a blood count *must* be accurate. That is why most technicians follow accepted techniques with utmost care . . . why they use only the finest equipment . . . why the majority choose the famous Spencer "Bright-Line" Haemacytometer.

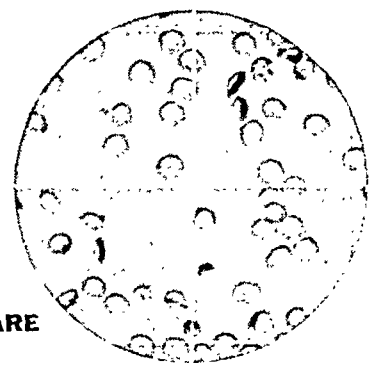
Only BRIGHT-LINE Chambers offer these 6 Safeguards to Accuracy

GREATER CONTRAST BETWEEN CELLS AND BACKGROUND

Cells appear dark, lines bright, background gray. Each cell is easily counted—even those which overlap lines.

MORE EVEN DISTRIBUTION OF CELLS

Surface tension differences between metalized surface of chamber and glass surface of cover cause cells to distribute more evenly and chamber to fill more readily.



SIMULTANEOUS RESOLUTION OF BLOOD CELLS AND RULINGS

It is not necessary to stop down the condenser of your microscope since blood cells and rulings can be clearly resolved at full diaphragm opening.

METALIZED BACKGROUND ELIMINATES GLARE

Absence of glare provides clearer vision and reduces eyestrain.

INDENTATION OF UNDERSIDE PROLONGS USEFUL LIFE

Introduction of a slight concavity directly under rulings prevents scratches appearing on lower surface of chamber. Such scratches impair counting accuracy.

PRODUCTION TOLERANCES ARE HELD TO THOSE RECOMMENDED AND SET FORTH BY THE U. S. BUREAU OF STANDARDS

American Optical
COMPANY
Scientific Instrument Division
Buffalo 15, New York

When you buy, specify "Bright-Line" and accept no substitutes. Immediate delivery from your supplier or write Dept. R16.

Makers of Medical Optical Instruments for Over 100 Years

SPENCER

MALLINCKRODT

sunlight, month after month



$AgNO_3$ $AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$
$AgNO_3$ $AgNO_3$

Silver Nitrate's traditional sensitivity to light is not inherent. It arises from various impurities. The purest Silver Nitrate, expertly made and carefully handled, withstands prolonged sunlight.

Lot after lot—not sometimes but always—Mallinckrodt Silver Nitrate A.R. withstands sunlight. It is consistently the fine reagent ACS specifications require.

Like all Mallinckrodt Analytical Reagents, Silver Nitrate A.R. assures you of the utmost Uniform Dependable Purity

ALWAYS SPECIFY MALLINCKRODT REAGENTS IN ORIGINAL PACKAGES

FINE CHEMICALS Mallinckrodt SINCE 1867

82 Years of Service to Chemical Users

MALLINCKRODT CHEMICAL WORKS

Mallinckrodt Street, St. Louis 7, Mo. • 72 Gold Street, New York 8, N. Y.

Chicago • Cincinnati • Cleveland • Los Angeles • Montreal • Philadelphia • San Francisco

UNIFORM DEPENDABLE PURITY

(In writing to advertisers, please mention the Journal—it helps.)

Announcing

ANTI-HUMAN PRECIPITIN SERUM

(rabbit)

for the "Coombs Developing Test"

a new and valuable diagnostic aid in

- Detection of infant sensitization with maternal Rh antibodies.[1,2,3,4]
- Detection of antibodies (blocking cryptagglutinoids) which coat but do not clump saline suspended cells.[1,3,4]
- Study and diagnosis of certain hemolytic anemias.[5]

packaging 2 cc vials with dropper cap assembly

a complete line of anti-rh sera now available

Ortho Anti-Rh Sera are carefully processed from human bloods and possess excellent avidity, specificity, and titer. Ortho currently offers:

- Anti-Rh_o (Anti-D) Slide Test Serum 4 cc pkg. (150 tests approx.)
- Anti-Rh_o (Anti-D) Test Tube Test Serum 2 cc pkg. (75 tests approx.)
- Anti-Rh_o' (Anti-CD) 87% Test Tube Test Serum 2 cc pkg. (75 tests approx.)
- Anti-rh' (Anti-C) 70% Test Tube Test Serum 2 cc pkg. (75 tests approx.)
- Anti-rh" (Anti-E) 30% Test Tube Test Serum 2 cc pkg. (75 tests approx.)

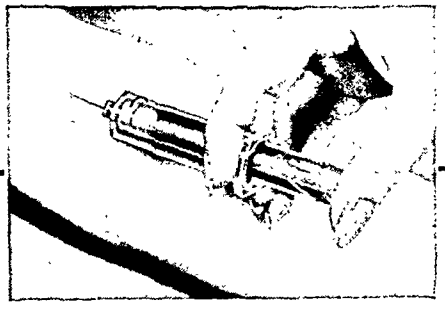
Further information and prices on request.

bibliography. (1) Coombs, R. R. A., Mourant, A. E., and Race, R. R.: A New Test for the Detection of Weak and "Incomplete" Rh Agglutinins, Brit. J. Exp. Path. 26:255-266, 1945. (2) Coombs, R. R. A., Mourant, A. E., and Race, R. R.: In-Vivo Isosensitization of Red Cells in Babies with Hemolytic Disease, Lancet, 250:264-266, 1946. (3) Hill, J. M., and Haberman, Sol: Demonstration of Rh Antibodies in the Newborn and Further Evidence of the Pathogenesis of Erythroblastosis, J. Lab. and Clin. Med., 31:1053-1066, 1946. (4) Levine, P., and Wigod, M.: Technic of Rh Testing, "Diagnostic Procedures and Reagents," Am. Publ. Health Assoc. In Press. (5) Sturgeon, P.: A New Antibody in Serum of Patient's with Acquired Hemolytic Anemia. Science, 106:293-294, 1947.

Ortho Pharmaceutical Corporation • Raritan, New Jersey

BLOOD SPECIMENS IN LESS THAN 7 SECONDS

without Hemolysis



when you use the

B-D **VACUTAINER®**

(A SPECIMEN TUBE)

AND NOTE THESE ADVANTAGES—

1 SPEED—10cc of Blood in less than 7 seconds—under normal conditions. Speed of Vacutainer may permit one technician to do the work of two using other methods.

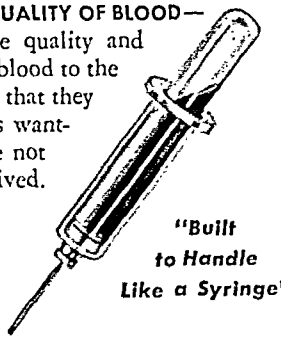
2 LOW COST—Original cost of equipment compares favorably with any other method. B-D Vacutainer saves cost of syringe, tube, cork, washing, scouring, sterilization, and other preparations for use. Less handling means less danger of breakage.

3 CLEANLINESS—Closed container eliminates contamination or possible spillage. Excess vacuum, after sufficient quantity of blood is taken into tube, automatically sucks residual blood from needle cannula into Vacutainer.

4 NO BLOOD TRANSFER NECESSARY — Blood is drawn from vein through needle into Vacutainer — where it remains for centrifuging and tests without need of transfer, also eliminating danger of outside contamination.

5 ADAPTABILITY—B-D Vacutainer tubes are available in a variety of sizes to fit most standard tests. They are supplied with or without anti-coagulant.

6 HIGH QUALITY OF BLOOD—Delivers the quality and quantity of blood to the laboratories that they have always wanted but have not always received.



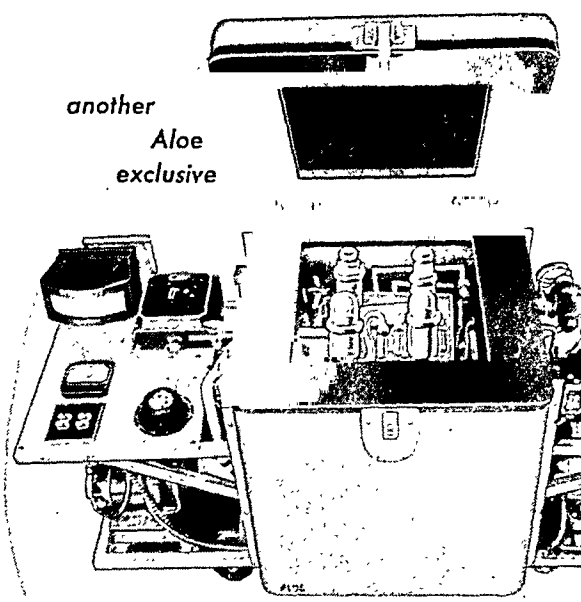
"Built to Handle Like a Syringe"

Literature on Request

B-D PRODUCTS

Made for the Profession

BECTON, DICKINSON & CO., RUTHERFORD, N. J.



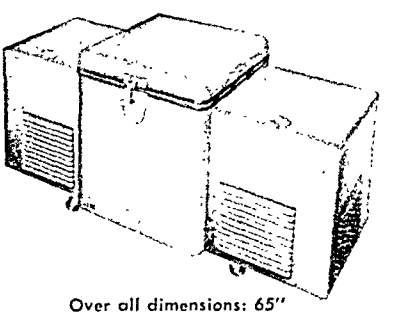
another Aloe exclusive

freezing-dehydration apparatus

for cell chemistry and pathology

New . . . Versatile . . . Compact. Built for exact studies. Aloe's flexible, stainless steel unit is based on the principles of the Altmann-Gersh freezing-drying apparatus and used by other recognized authorities. Important engineering improvements include direct reading scales and manual controls of both temperature and vacuum.

Under accurate controls, the new Aloe Freezing-Dehydration Apparatus, permits tissue to be dehydrated and fixed with minimal losses and alterations. Tissue, after freezing in isopentane, is placed in one of the vacuum chambers in refrigerating compartment. Temperature is accurately adjusted from —15°C to —50°C and vacuum as low as 0.001 mm mercury. If desired, the specimen is infiltrated with paraffin or cellodin. Sections of embedded materials may be used for morphological and cytochemical studies by numerous methods.



Over all dimensions: 65" wide x 28½" deep x 34½" high with covers closed and 56½" high with covers open.

71580 FREEZING—DEHYDRATION APPARATUS, as described, with four vacuum-dehydration chambers, two desiccant tubes, and one Dewar flask. For 115 volts, 60 cycles, A. C. each.....$2,250.00

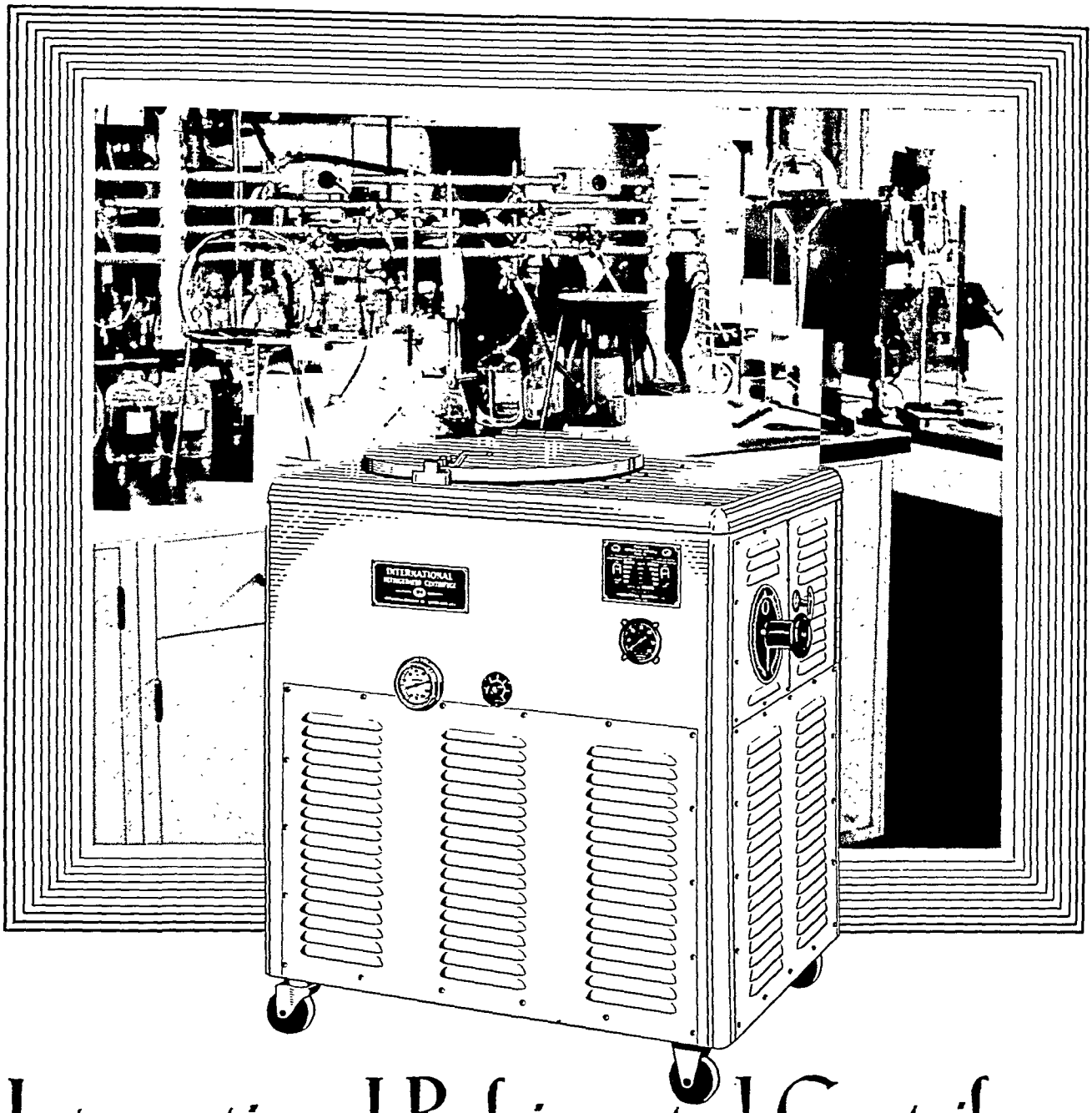
Write for illustrated booklet and bibliography — Bulletin No. T112

A. S. ALOE COMPANY *Serving science since 1860.*

1831 Olive Street • St. Louis 3, Missouri

aloe

(In writing to advertisers, please mention the Journal—it helps.)



International Refrigerated Centrifuge

Centrifugation under controlled temperatures at or well below 0° C. offers new possibilities to the biochemist. The cancer program —virus investigations—poliomyelitis research —enzyme research—low temperature solvent fractionation—all have benefited by the use of this new research tool. Countless other applications are waiting the guidance of the research scientist.

The International Portable Refrigerated Centrifuge, Model PR-1, is now equipped with a larger capacity compressor. Centrifuge speeds and capacities remain unchanged, but much lower controlled temperatures are now possible. For example, with six 100 ml. tubes at 5000 r.p.m., temperatures as low as -15° C. can be maintained; with four 250 ml. bottles at 2600 r.p.m., -8° C.; with four 25 ml. tubes at 18,000 r.p.m., -5° C.

Here is a scientifically designed research instrument definitely worth investigating. Write for descriptive Bulletin RC, 1949.

INTERNATIONAL EQUIPMENT COMPANY
Boston 35, Massachusetts

C. I. No. 331

BISMARCK BROWN Y

. GM5

Certified for use in: Histology, Bacteriology (Staining)

Pharmaceutical Laboratories

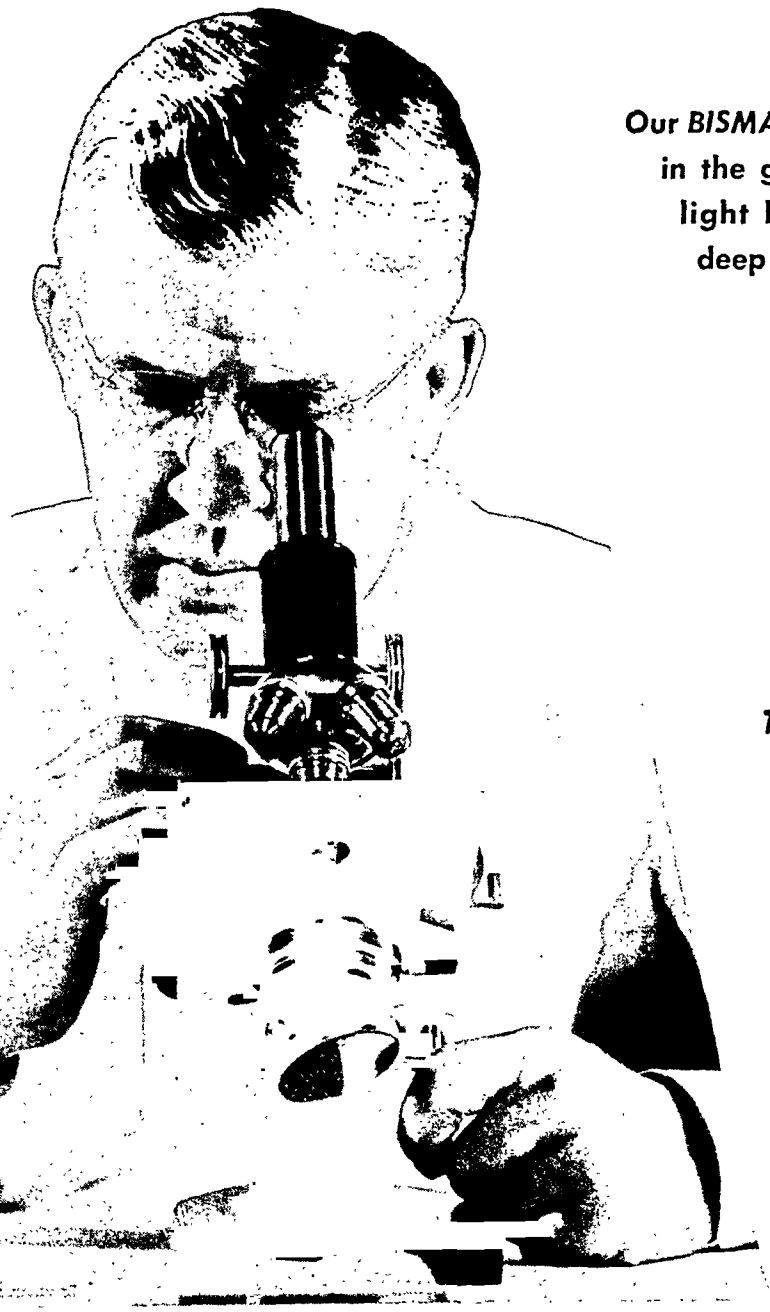
NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A.

Found satisfactory by BIOLOGICAL STAIN COMMISSION *for above-mentioned purposes. This does not imply approval for any medicinal use. Will users please report any unsatisfactory results to H. J. Conn, Geneva, N. Y.*

Total Dye Content %

Certification No.
NN



Our *BISMARCK BROWN Y* stains mucous in the goblet cells of the intestine a light brown, contrasting with the deep brown cartilage.

National

THE COMPLETE LINE OF
COMMISSION CERTIFIED BIOLOGICAL STAINS

NATIONAL N COMMISSION PHARMACEUTICALS

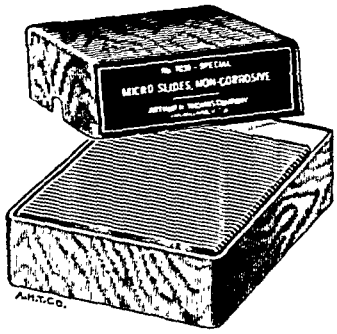
NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A.

A.H.T. CO. SPECIFICATION

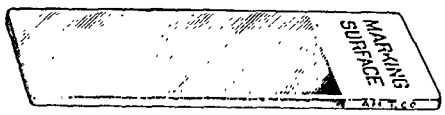
MICRO SLIDES

NON-CORROSIVE, RED LABEL



7030.

Of hard glass, nearly pure white in color and guaranteed against corrosion in any climate.



7036-A.

MICRO SLIDES, Non-Corrosive, Red Label, A.H.T. Co. Specification. Of hard glass, nearly pure white in color and guaranteed against corrosion in any climate. Thickness varies between 1.18 and 1.30 mm, with an average of 1.23 mm.

The glass from which our Slides are made is of superior quality—particularly as to surface finish and uniform thickness—so that the two grades are identical as to stability, optical quality and planeness of surface, the following classification depending upon freedom from bubbles, pits, nicks and striae and in the finish of the edges:

Special Slides. With polished edges, slightly beveled on the three-inch edge, and with rounded corners. Entirely free from bubbles, pits, nicks and striae. Individually selected and offered as, in our judgement, *the best micro slide now available.*

Clinical Slides. With ground edges and rounded corners, otherwise identical with Special slides except for the omission of the slight bevel on the three-inch edge and that selection is based upon the plate from which the slides are cut, rather than upon the individual slide, so that an occasional slide may be found with a slight defect. Offered as *a superior slide for general work.*

Some Micro Slides show cloudiness near the edge—and sometimes throughout the usable surface—as a result of the deposit of powdered glass in solution from the edge-grinding process, with too rapid drying and insufficient cleaning thereafter. Our Slides are free from this defect because of the procedure followed for cleaning and drying after manufacture.

7030. **Micro Slides, Special, Non-Corrosive, Red Label** size 3 × 1 inches, as above described. Packed in distinctive brown grained cartons containing ½ gross. **Per gross**... 2.05

7030-B. **Ditto, Clinical. Per gross**..... 1.65

10% discount in case containing 25 gross, one catalogue number only
20% discount in lots of 100 gross } 7030 and 7030-B, assorted
25% " " " " 500 " } in 25 gross case units

7036-A. **Micro Slides, Special, Non-Corrosive, Red Label,** size 3 × 1 inches, identical with No. 7030 but with 18 mm surface at one end etched evenly on *both* sides for marking. This etched surface has a superior velvet matte finish which takes lead pencil, hard wax pencil, India ink, "Labink" or similar preparations, and ordinary writing ink much better than the usual ground surface. **Per gross**..... 3.00

7036-B. **Ditto, Clinical. Per gross**..... 2.60

10% discount in case containing 25 gross } one catalogue
20% " " lots of 100 gross } number only

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

CABLE ADDRESS, "BALANCE," PHILADELPHIA

CYTOLOGIC DIAGNOSIS OF UTERINE CANCER BY EXAMINATION OF VAGINAL AND UTERINE SECRETIONS*

GEORGE N. PAPANICOLAOU, M.D.

From the Department of Anatomy, Cornell University Medical College, New York, New York

Mr. Chairman, members of the American Society of Clinical Pathologists, and guests.

I appreciate very deeply the honor of participating in this meeting and the privilege of discussing with you a problem to which I have devoted so many years of my life, that of exfoliative cytology, and more specifically, that part of it which applies to the diagnosis of uterine cancer through the examination of vaginal and uterine secretions.

Since 1943 when Dr. Herbert F. Traut and I published our monograph on the Diagnosis of Uterine cancer by the Vaginal Smear,[1] there has been a revival of interest in this method of diagnosis. I say "revival" because the idea of examining various fluids of the body for the purpose of detecting cancer cells is not new.

The recognition which this method is now receiving is, of course, most gratifying and I am particularly happy that pathologists are taking more and more interest in it. I feel that this is a method which will finally be entrusted to them and that its future depends chiefly upon their attitude toward it and their acceptance of it as a *bona fide* laboratory procedure.

Unfortunately, the introduction of a new technic such as this, which requires a relatively long training period, is bound to proceed slowly because of the fact that for some time to come there will undoubtedly be a shortage of adequately trained laboratory men to handle this type of work. A special training period of at least three months, preferably one of from four to six months, in a properly qualified laboratory, is to be recommended, even for those with a good pathologic background, for acquiring sufficient skill in technic and interpretation.

In spite of the progress that has been made in the use of the vaginal smear during the last few years, we have not yet reached a point where we can consider it as a method of final diagnosis. Much pioneer work remains to be done. Anyone visiting our little exhibit and getting a glimpse of the amazing variety of normal and abnormal cells which may be encountered in the different body fluids will realize the magnitude of the task which lies before us. It will take many years before we reach a point from which we can evaluate with greater accuracy and confidence these many cell forms. Until we do so it would seem advisable to exercise great caution and to recommend, whenever possible, confirmation of smear findings with biopsy of curetted or excised tissue.

The technic used in our work has been fully described in previous papers,[2, 3, 4] so I feel that I can dispense with a detailed description here. I only want to

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, December 9, 1948.

emphasize the importance of immediate fixation and proper staining. Our staining procedure is not specific for cancer cells, but only adapted to cancer diagnosis. It consists of the staining of the nucleus with Harris' Hematoxylin and counterstaining of the cytoplasm with Orange G and EA36 or EA50 which insure a light and transparent coloration of the cytoplasm and a good differentiation between basophilic and acidophilic cells. A very deep staining of the nucleus should be avoided as it may give a false impression of hyperchromasia.

With regard to the type of smear, we still give first place to smears taken by aspiration. We request, first of all, a vaginal smear taken before the introduction of a speculum. Such a smear gives a more accurate idea of the extent of normal exfoliation and of the presence of any bleeding not due to trauma. It also permits better evaluation of the prevailing bacterial flora and the detection of any existing parasites. Furthermore, in an aspiration vaginal smear one might expect to recover exfoliated cells from the vagina, ecto- and endocervix as well as from the endometrium.

Our second request is for a cervical, or rather endocervical, aspiration smear which gives a better picture than does the vaginal smear, of the cytology of the endocervical fluid and usually a larger representation of endometrial cells. It is particularly valuable in cases of early carcinoma of the cervix, in which, as a rule, one finds a larger number of cancer cells in the endocervical than in the vaginal smear.

Our third request is for a direct smear taken either with a cotton swab or with a wooden spatula such as that designed by Dr. Ernest Ayre.[1] In lesions occurring near the cervical os this type of smear offers the great advantage of procuring a larger number of malignant cells. However, should one be limited to the swab smear alone, there would be the risk of missing the malignant cells coming from the upper part of the endocervical canal as well as from the endometrium and the tubes. I have repeatedly seen cases in which the endocervical aspiration smear contained endometrial adenocarcinoma cells whereas the cervical swab smear was entirely negative. Another disadvantage is that cervical swab smears, being more cellular, tend to dry faster than aspiration smears and often lead to an erroneous interpretation. Drying causes enlargement of the cells and their nuclei, which is often misleading.

A fourth type of smear which is most desirable is the endometrial aspiration smear. It is extremely valuable in cases in which an endometrial malignant growth is suspected.

In cases where all four types of smears are available the chances of missing a malignant lesion are greatly reduced. However, only rarely may one expect to receive all four types. In most instances the material coming to us consists chiefly of vaginal aspiration and cervical aspiration or swab smears.

Most of the statistics which are available at present are based on these two types of smears, chiefly on vaginal aspiration and to a lesser extent on cervical aspiration or swab smears. Differences in the types of smears partly account for the discrepancies found in statistics published to date. Strictness of criteria, skill and experience, careful screening and adequate technic are other factors

which may affect statistical data. Discrepancies would also be expected to exist in comparisons of different groups of patients, as those examined in cancer prevention clinics who, for the most part, are asymptomatic and those examined in private practice or in hospital clinics most of whom have symptoms.

Discrepancies such as these are shown in a statistical evaluation of some of our more recent cases, representing two different groups, one consisting of 124 patients examined at the Woman's Clinic of the New York Hospital, the other of 777 patients referred by private physicians. In the first group of patients which included only those with symptoms, the incidence of uterine cancer was relatively high, totaling 20 (16.1 per cent). In the second group of patients most of whom were asymptomatic and relatively few with symptoms, the incidence of uterine cancer was much lower, totaling 11 (1.4 per cent).

Two of the 20 positive cases in the first group (all proved by biopsy) had been reported by us as negative, which gives 10 per cent of false negatives. These two were cases of early carcinoma of the cervix, one of a carcinoma *in situ* and the other a squamous cell carcinoma grade 1 (Broder's grading of tumors).* There were no false positives in this first group.

In the second group one of the 11 positive cases (all proved by biopsy) had been reported as negative, which gives 9.1 per cent of false negatives. This one was also an early carcinoma of the cervix, a carcinoma *in situ*. Three of the 766 negative cases had been reported as positives, two as Class IV and one as Class V (author's cytologic classification), which gives 0.4 per cent of false positives. Of these, two were found to have cervical metaplasia, and one chronic cervicitis with leukoplakia.**

A recent statistical evaluation of 3195 cases examined at the Kate Depew Strang Cancer Prevention Clinic† shows a still lower incidence of uterine cancer, a total of 10 (0.3 per cent), as proved by biopsy. In this series there were no false negative reports and only one false positive,‡ giving a percentage of 0.03.

These figures give a general idea of the results which may be expected at the present stage of development of the smear method and of the differences existing between varied groups of patients. In most of the above cases two types of

* In the above evaluation we take for granted that the final pathologic diagnosis is the correct one, in spite of the fact that there has been at times a question in our minds as to the accuracy of the interpretation of the biopsy. In all instances in which there has been a discrepancy between the interpretation of a smear and that of a biopsy we always gave the benefit of the doubt to the latter, although we have had cases in the past in which the interpretation of the biopsy was later proved to be erroneous.

** None of these three cases was explored by serial sections. Therefore, one cannot entirely exclude the possibility of the presence of a small cancerous lesion which remained undetected.

† These cases have been examined since October 1, 1947, when I was entrusted with the supervision of the cytologic laboratory of the Clinic. They represent unpublished data.

‡ The interpretation of the smear findings in this case as a false positive was based on a negative cervical biopsy. A subsequent biopsy, performed since these results were tabulated, proved the existence of an early squamous cell carcinoma, most likely pre-invasive. In the light of this fact the evaluation of this group should be corrected to read as having 100 per cent accuracy.

smears were available, the one vaginal aspiration, and the other cervical aspiration or cervical swab.

It is to be noted that our results with adenocarcinomas of the endometrium or of the cervix are much better than those previously reported.[4] In the first group of 124 cases there were 4 adenocarcinomas of the endometrium and 1 of the cervix, and in the second group of 777 cases there were 5 adenocarcinomas of the endometrium and 1 of the cervix. All were diagnosed correctly by the smear. This contrasts with a 9.3 per cent of false negatives reported by Dr. Traut and myself in our 1943 monograph.

The criteria used in the cytologic diagnosis of cancer are based on structural modifications of the cancer cells and of their nuclei as well as on changes in their relationship and pattern when in groups and clusters. In the early stages of our work our diagnostic objective was limited to a decision as to whether malignancy was or was not present. Increased knowledge and experience are now enabling us to recognize with greater confidence certain characteristic cell or smear types which in some instances make possible an evaluation of the type of the tumor as well.

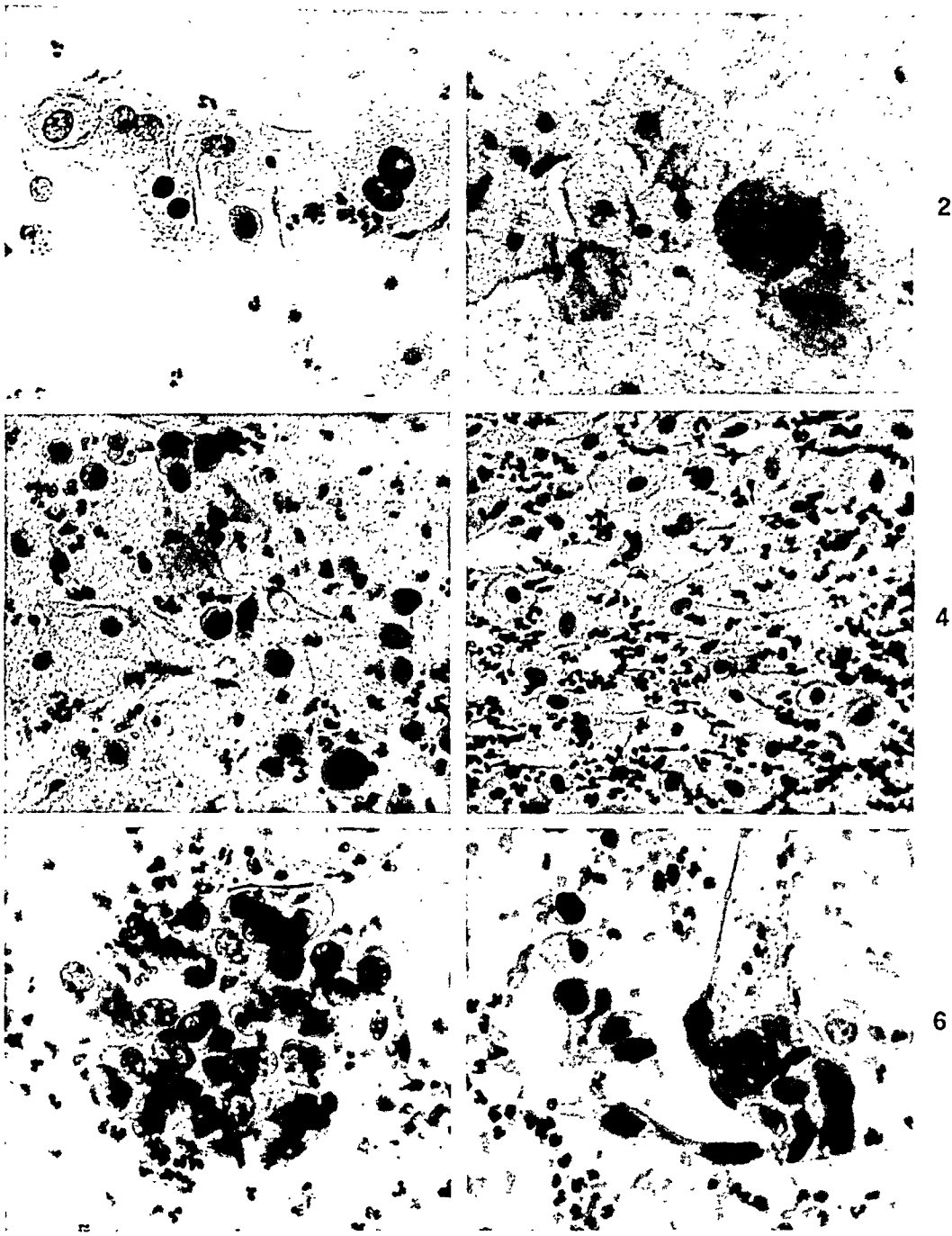
One such smear type is what we refer to as "superficial dyskaryosis".* Smears of this type are characterized by the presence of marked nuclear abnormalities, which are, however, limited chiefly to the superficial squamous cells (Fig. 1; compare with Fig. 2). The abnormal nuclei are enlarged and hyperchromatic. Binucleated or even multinucleated cells are not uncommon. A characteristic perinuclear vacuole is often to be seen. Some of the abnormal cells show cornification and are distinctly acidophilic. Eventually the nuclear atypia may be extended to cells of the parabasal layers.

In cases exhibiting this smear type a high incidence of early carcinoma of the cervix, more specifically of the intra-epithelial type, has been found to exist.† However, in a large percentage of such cases biopsies have been persistently negative, and in one case at least which has been under observation for approximately 10 years, this condition was apparently reversible, as indicated by the complete disappearance of abnormal cells from later smears. We, therefore, consider cases of this type as strongly suggestive of an early localized low grade malignant neoplasm of the cervix, which may remain dormant over a long period of time or may even undergo regression. A high percentage of parasitic infections, chiefly trichomoniasis, has been noted in such cases. In a few instances cells of the "dyskaryosis" type were found intermixed with typical cancer cells in smears of cases which proved to be advanced invasive squamous cell carcinomas of the cervix. This seems to indicate that eventually this condition may either become associated with or develop into a frank cervical carcinoma.

The cytology of this type of lesion corresponds closely to what is known as dyskeratosis of the skin, yet this term could not be applied to it very well be-

* In previous discussions this condition was referred to as Birkhead's disease. A short description of it is given on page 31 and an illustration in plate D, Figure 1 of our 1943 monograph.[4]

† A more complete description together with a statistical evaluation of cases of this type is now being prepared for publication.



All figures: stains used, hematoxylin, OG 6, EA 50, procedure 268; magnification, × 330.

FIG. 1. Vaginal smear. "Superficial dyskaryosis". Cluster of superficial squamous cells showing nuclear enlargement with hyperchromasia, binucleation, and a perinuclear vacuole.

FIG. 2. Vaginal smear. Cluster of normal superficial squamous cells. Note normal nuclei.

FIG. 3. Vaginal smear. Carcinoma *in situ*. Note scattered carcinoma cells of parabasal type with much enlarged and hyperchromatic nuclei.

FIG. 4. Vaginal smear. Menopausal atrophic type. Note parabasal cells with normal nuclei.

FIG. 5. Vaginal smear. Squamous cell carcinoma of cervix. Cluster of malignant cells.

FIG. 6. Vaginal smear. Squamous cell carcinoma of cervix. Clusters of malignant cells. Note more differentiated epidermoid cells.

cause of the absence of typical keratinization. Further study is necessary to determine the nature and significance of this distinctive cytologic picture.

Another smear type frequently associated with early carcinomas of the cervix, more particularly of the intra-epithelial pre-invasive type, is the one shown in Figure 3 (compare with Fig. 4). In this there is a prevalence of abnormal cell forms of the parabasal type, mostly round or oval cells with a disproportionately enlarged and activated nucleus which tends to be distinctly hyperchromatic. There is considerable variability in the size of the cells, which appear singly, scattered throughout the smear, or in small groups, but not in the large impressive clusters characteristic of advanced squamous cell carcinomas. Cervical parabasal cells are usually numerous and some show pronounced vacuolization. One is often tempted to venture a diagnosis of a carcinoma *in situ* on the basis of a smear of this type although the presence or absence of invasion can never be decided on the basis of a smear study alone.

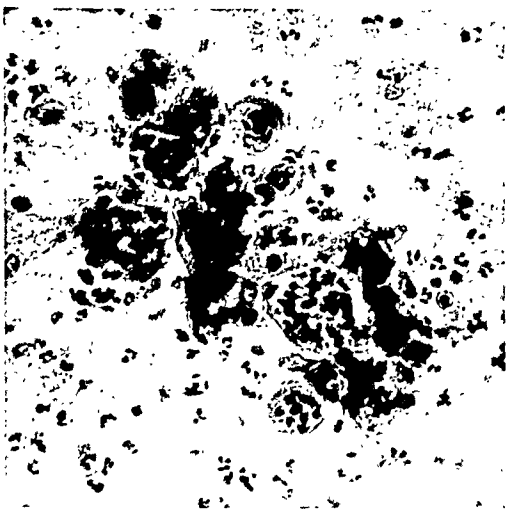
Squamous cell carcinomas of the cervix are characterized by a larger variety of more differentiated abnormal cells which often gather into dense groups (Figs. 5 and 6). Nuclear enlargement and activation, hyperchromasia, irregular fragmentation of the nucleus, engulfment, anisocytosis and anisokaryosis are more frequently encountered. Some cells exhibit extremely atypical and bizarre forms. Marked elongation of the cells is characteristic of well-differentiated epidermoid cancerous lesions.

Adenocarcinomas of the endometrium or of the cervix present a different pattern. The malignant cells are of the glandular type and are generally smaller than the squamous cells of the cervix. Vacuolization is more pronounced and not infrequently the vacuoles contain small cells mostly of the leukocytic type (Fig. 7). The nuclei show enlargement and hyperchromasia and are often eccentrically located. The cells tend to form dense clusters with an irregular pattern and anisokaryosis. Necrotic cells or stripped nuclei with an irregular grouping of coarse chromatin granules of unequal size, or single cells with an enlarged eccentric nucleus and inclusions of degenerating leukocytic elements are almost always present and are of great diagnostic value.

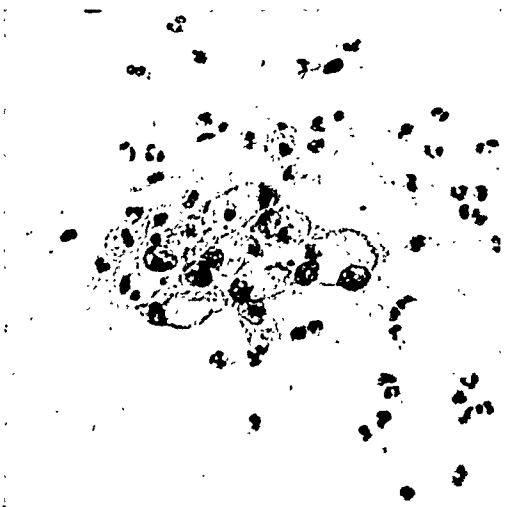
Adenocarcinomas of the cervix have a comparable cytology although the cells tend to be larger. Their differentiation from adenocarcinomas of the fundus is rather difficult unless some of the cells retain their well-defined mucoid type, which permits their identification (Fig. 8).

A smear type with distinctive though not malignant cytology is that of endometrial metaplasia (Fig. 9). This is characterized by the presence of cell clusters of densely grouped normal endometrial cells, the more peripheral ones of which show enlargement and marked vacuolization or distinct metaplasia. The vacuoles are usually empty and only rarely contain leukocytic inclusions. Such cell groups bear a marked resemblance to clusters of adenocarcinoma cells but may be distinguished from them by their normal and uniform nuclei and by the characteristic peripheral arrangement of the larger vacuolated or metaplastic cells.

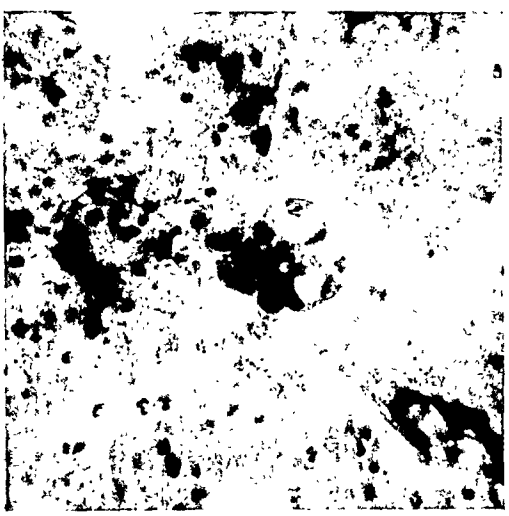
Adeno-acanthomas of the endometrium or of the cervix also have a cytology of their own. Cells revealing marked squamous metaplasia and an abnormal



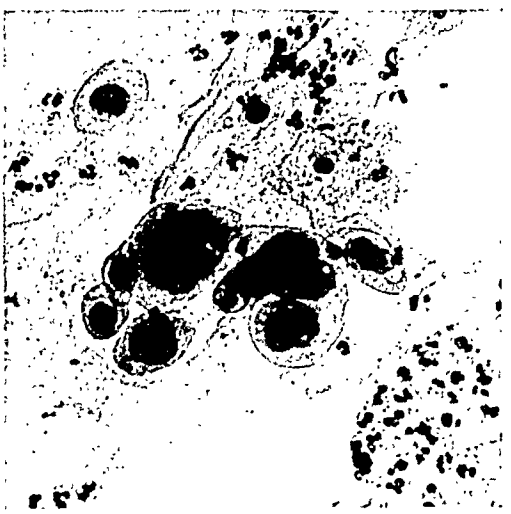
7



8



9



10

All figures: stains used, hematoxylin, OG 6, EA 50, procedure 268; magnification, × 330.

FIG. 7. Vaginal smear. Adenocarcinoma of endometrium. Cluster of malignant cells. Note invasion of some of the cells by leukocytes.

FIG. 8. Cervical smear. Adenocarcinoma of cervix. Cluster of malignant cells. Note mucoid type of cells.

FIG. 9. Vaginal smear. Metaplasia of endometrium. Cluster of endometrial cells. Note three enlarged vacuolated cells located at periphery of a group of normal endometrial cells.

FIG. 10. Vaginal smear. Adeno-acanthoma of endometrium. Cluster of malignant cells. Note metaplasia and nuclear atypia.

nuclear structure are the most characteristic cellular elements of this type of lesion (Fig. 10). Their differentiation from cells of the squamous cell carcinoma type is often very difficult.

The above short descriptions give a general idea of some of the characteristic smear types which may be found to be associated with various types of carcinomas of the cervix and of the endometrium.

In closing, I should like to emphasize the value of the cytologic method as a useful procedure not only in the diagnosis of cancer, particularly in the early carcinomas of the cervix and the endometrium, but also as a method which may supply us with very valuable material for the study of the early pathogenesis of malignant neoplasms.

REFERENCES

1. AYRE, J. E.: Selective cytology smear for diagnosis of cancer. Am. J. Obst. and Gynec., **53**: 609–617. 1947.
2. PAPANICOLAOU, GEORGE N.: New procedure for staining vaginal smears. Science, **95**: 438–439, 1942.
3. PAPANICOLAOU, GEORGE N., AND MARCHETTI, ANDREW A.: The use of endocervical and endometrial smears in the diagnosis of cancer and of other conditions of the uterus. Am. J. Obst. and Gynec., **46**: 421–422, 1943.
4. PAPANICOLAOU, GEORGE N., AND TRAUT, HERBERT F.: Diagnosis of Uterine Cancer by the Vaginal Smear. New York: The Commonwealth Fund, 47 pp., 1943.

CYTOLOGIC DIAGNOSIS OF CANCER FROM PLEURAL AND PERITONEAL FLUIDS*

OTTO SAPHIR, M.D.

From the Department of Pathology,† Michael Reese Hospital, Chicago, Illinois

Wihman[5] in 1948, in a special supplement to the Acta medica Scandinavica, gives a historical resumé of our knowledge of the interpretation of the cellular contents of exudates and transudates. For the purpose of this discussion, it may be pointed out that as early as 1867, Lücke and Klebs[2] reported the presence of tumor cells in exudates. Quincke[3] a few years later drew attention to the fact that tumor cells in exudates are often aggregated in clumps. He described quite accurately the morphologic appearance of such tumor cells. After the introduction of the more modern staining methods by Ehrlich,[1] smear diagnosis was made with greater certainty.

Because of certain difficulties which were bound to arise in these early days, pathologists reverted to histologic rather than cytologic methods. Schmorl,[4] as early as 1918, described the method of centrifuging exudates in alcoholic solutions and treating the exudate as curetted specimens, eventually placing the sediment in paraffin and cutting sections. Numerous pathologists have used this method since the early twenties and have found it quite satisfactory. The important objection to this method is the time element, since these very small particles have to be run through the various solutions carefully and have to be handled with utmost caution. Thus, it takes several days before slides are available for diagnosis. Besides, if the sediment is minute, one simply may not be able to embed it. Smears from sediments can be examined much more quickly and even the most minute amount of sediment is suitable for smears. Usually the diagnosis can be made on the same day the specimen is received. The most important step in this procedure is the immediate fixation of smears of the sediment in an alcohol and ether mixture.

In the following discussion, an analysis of examinations of 175 pleural or peritoneal effusions will be given and a few pitfalls in the diagnoses of malignant tumor cells pointed out.

The fluid, whether it be of pleural or peritoneal origin, should be examined as soon as it is collected. In the event of unavoidable delay, the fluid should be placed in a refrigerator until it can be delivered to the histologic laboratory. The full amount of fluid and not "samples" of fluid should be sent to the laboratory. The fluid is then centrifuged at about 1700 r.p.m. for about ten minutes. After the supernatant fluid is decanted, the sediments from the various centrifuge tubes are finally collected into one tube and a pooled sediment obtained. Smears

* Presented at the Twenty-Seventh Annual Meeting of American Society of Clinical Pathologists, in Chicago, October 13, 1948.

This work was aided by a grant from the Ira Frank Fund.

Received for publication, December 13, 1948.

† This department is, in part, supported by the Michael Reese Research Foundation.

are made from this sediment with the use of a "platinum" loop. The glass slides are prepared by the usual method of cleaning and then coated with a thin layer of egg albumin. In making the smears, fragments of tissue or other masses which may contain the tumor cells are selected. Attempts should be made to obtain even smears that are not too thick. The smears are then gently placed in the ether-alcohol fixative (which consists of equal parts of ether and 95 per cent ethyl alcohol) while still wet. In order to keep the slides from adhering to each other, a paper clip may be attached to one end of each slide. The slides are kept in the fixative for 20 to 30 minutes before they are stained. The smears can be kept in the fixative for an almost indefinite period, if necessary.

For this study, the slides were stained routinely according to the Papanicolaou method. Whenever enough material was available, the hematoxylin-eosin staining method and the Giemsa stain were also used. It may be mentioned here that hematoxylin and eosin also gave very good results. The following technics were used for staining the slides.

Papanicolaou's Staining Method

After fixation in ethyl alcohol and ether mixture, immerse slides successively in 80 per cent, 70 per cent, 50 per cent ethyl alcohol and distilled water.

Stain in Harris' hematoxylin for two minutes.

Rinse in distilled water.

Dip in 0.5 per cent hydrochloric acid (aqueous solution) to destain.

Place in running tap water for five minutes, or until nuclei appear blue.

Rinse in distilled water.

Immerse successively in 50 per cent, 70 per cent, 80 per cent and 95 per cent ethyl alcohol.

Stain in OG 6 (Orange G-0.5 per cent solution in 95 per cent ethyl alcohol) for two minutes.

Rinse in 95 per cent ethyl alcohol, two changes.

Stain in EA 36 (Bismarck brown, eosin yellowish and light green yellowish) for one-half minute.

Rinse in 95 per cent ethyl alcohol (three changes); absolute ethyl alcohol and xylol, equal amounts, xylol (two changes) and mount with balsam.

Giemsa's Staining Method

After fixation in ether-alcohol, rinse slides in 95 per cent ethyl alcohol and then in three or four changes of distilled water.

Allow slides to remain in the Giemsa stain (1 drop concentrated Giemsa stain per cc. distilled water) for at least one-half hour.

Rinse in three changes of distilled water.

FIG. 1. Multinucleated mesothelial type of giant cells. Papanicolaou's staining method. × 275.

FIG. 2. Mesothelial cell in mitosis. Papanicolaou's staining method. × 300.

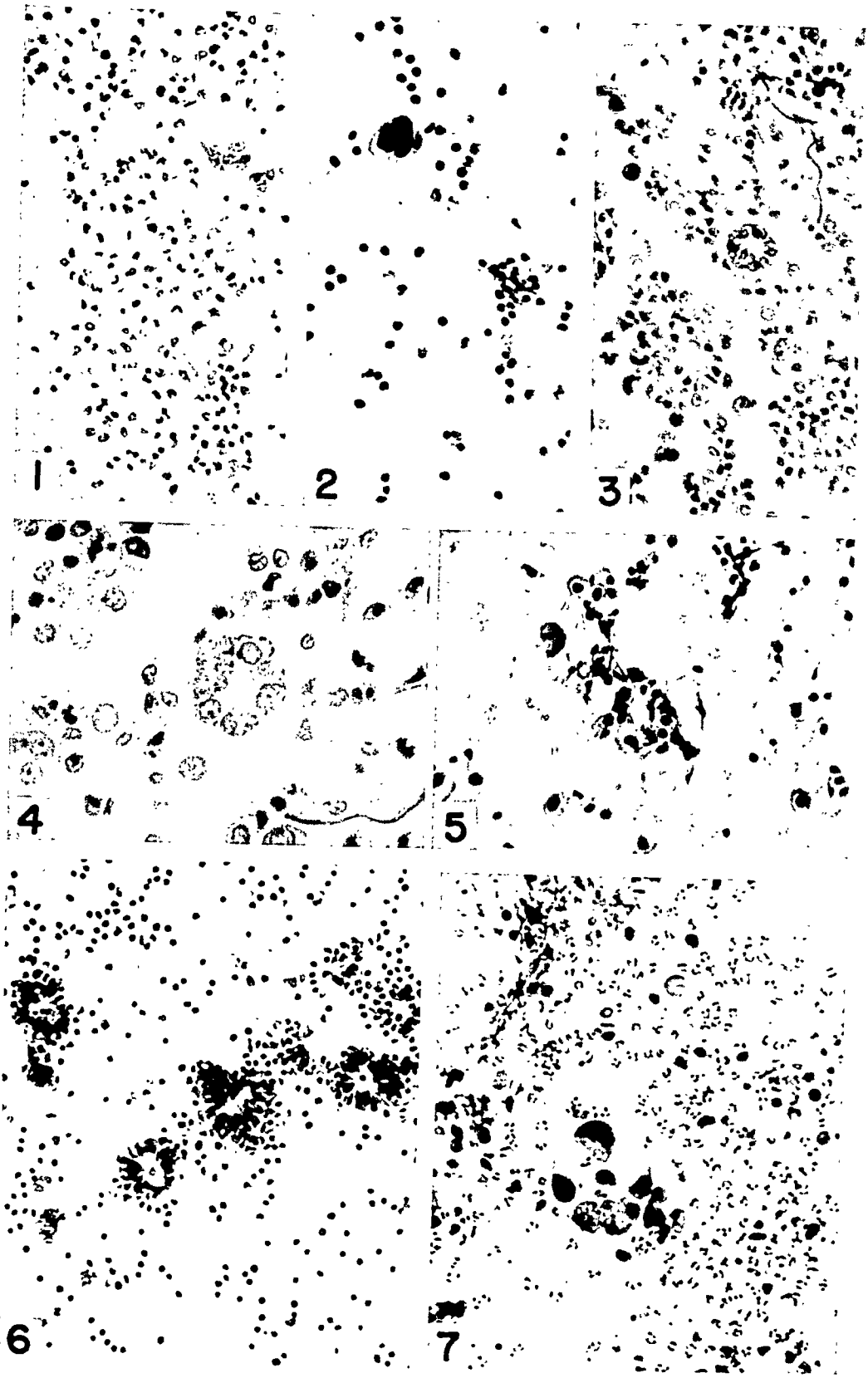
FIG. 3. Pseudo-glandular structure in ascitic fluid from a patient with portal cirrhosis. Iron-hematoxylin and eosin preparation of paraffin section of sediment. × 350.

FIG. 4. From same field as Figure 3. Iron-hematoxylin and eosin preparation of paraffin section of sediment. × 650.

FIG. 5. Pseudo-glandular structure in peritoneal exudate. Papanicolaou's staining method. × 350.

FIG. 6. Adenocarcinomatous structure. Iron-hematoxylin and eosin stain of a smear. × 275.

FIG. 7. Multinucleated carcinoma cell in peritoneal exudate. Papanicolaou's staining method. × 650.



FIGS. 1–7

Rinse in two changes acetone.

Rinse in two changes xylol and examine smears at this point. If necessary to destain further, retrace steps back to distilled water and then continue as before.

Mount slides from the second change of xylol with balsam.

Hematoxylin and Eosin Staining Method

After fixation in ether-alcohol, run slides down through 80 per cent, 70 per cent, and 50 per cent ethyl alcohol to distilled water as done for the Papanicolaou staining method.

Stain in Harris' hematoxylin for two minutes.

Rinse in distilled water.

Destain in 0.5 per cent aqueous solution of hydrochloric acid.

Place into running tap water for five minutes or until nuclei are blue.

Stain in eosin for one-half minute.

Rinse in three changes of 95 per cent ethyl alcohol.

Allow slides to remain in carbol-xylol for five minutes.

Rinse in two changes xylol and mount with balsam.

In this study of the slides of 175 effusions, 73 of the specimens were from the pleural cavity and 102 were of peritoneal origin.

Whenever sufficient sediment was obtained, parts of the sediment were also prepared for embedding in paraffin and cut as tissues. Thus, often comparisons could be made between histologic and cytologic methods. This dual examination is to be recommended especially for those who seek experience in studying individual cells in smear preparation. After comparing both methods, it became clear that the cytologic smear method is superior.

The most common type of cellular elements found in routine specimens were red blood corpuscles, polymorphonuclear leukocytes, round cells which are most commonly lymphocytes, and desquamated mesothelial cells. The characteristic features of such mesothelial cells are the clear-cut cytoplasm, which takes a pale eosinophilic stain and the well outlined, usually perfectly round nucleus, with scarcely recognizable nucleolus. Sometimes some of these cells are large and contain two or three nuclei. Such multinucleated giant cells have round nuclei, all of about the same size and shape and are obviously identical to those found in mononuclear mesothelial cells. They have a large, somewhat ill-defined eosinophilic cytoplasm which is perfectly homogeneous. These giant cells are not indicators of malignancy. Particularly in instances of cirrhosis of the liver of long duration are normal mesothelial cells and giant cells found in abundance in the sediment. Also often found in these instances are mesothelial cells arranged in clumps or heaps, and isolated groups and clusters of these cells may be noted in the smear. It must be emphasized that such clumps are definitely not a sign of malignancy.

Some of these cells may be arranged in ringlike fashion and may assume the shape of a tubule. When such pseudo-glandular structures were found first, they were thought to be evidence of adenocarcinoma. Only by experience was it learned that these structures are not true glandular structures, and that they are similar to those occasionally encountered in paraffin sections of infolded visceral pleura or peritoneum, formed by rows of swollen mesothelial cells. A careful study of these structures shows that the individual cells of which these pseudo-

glands are composed have a small nucleus with minute or not recognizable nucleoli and an eosinophilic cytoplasm. Close inspection reveals that the majority of these cells and nuclei are alike and are morphologically similar to mesothelial cells. They do not vary in size, shape or staining quality. Mitotic figures are often encountered in these cells. As just stated, these structures when first seen were thought to be evidence of malignancy, and as a matter of fact, a false-positive diagnosis was made from the exudate, based on the findings of such structures.

Cells in mitosis are not characteristic of malignancy. Mesothelial cells apparently multiply quite often by mitosis. Only outspoken atypical mitoses, such as the presence of three spindles, or asymmetrical spindles may be taken as evidence of malignancy. As a matter of fact, in malignant neoplasms involving serous membranes, highly atypical mitotic figures are often encountered and usually can be demonstrated.

The outstanding characteristic of malignant tumor cells in individual smears is the large, sometimes irregular nucleus which is often twice the size of the

TABLE 1

SUMMARY OF FINDINGS IN CYTOLOGIC EXAMINATION OF SMEARS FROM 175 PLEURAL OR PERITONEAL EFFUSIONS

	NUMBER
Pleura or peritoneum involved by carcinoma	45
Carcinoma correctly diagnosed	37
Carcinoma present but diagnosis "doubtful"	6
Carcinoma present but not diagnosed (false negative)	2
Carcinoma absent but smear diagnosed as showing carcinoma (false positive)	1

nucleus of mesothelial cells. Vacuoles in the nuclei and relatively large nucleoli are common. The cytoplasm is correspondingly larger in amount than normal, but offers no unusual characteristics. The diagnosis of malignancy in these preparations rests solely on the observation of the nucleus in malignant cells, its size, the presence of large nucleoli, and the findings of outspoken atypical mitoses.

Following such criteria, carcinoma cells were reported 37 times in smears of the 175 effusions. These findings proved to be correct later at autopsy. In one instance, a smear taken from a peritoneal exudate was diagnosed as positive for carcinoma cells. At autopsy in this instance, cirrhosis of the liver was found, but there was no evidence of carcinoma (false positive). This diagnosis was based on the finding of pseudo-glandular structures. A negative diagnosis (false negative) for cancer was made twice. In one of these instances, the patient subsequently died of carcinoma of the stomach and in the other, the patient died of carcinoma of the colon. Both patients had peritoneal metastases and a serosanguineous (carcinomatous) peritonitis. Six smears taken from patients who subsequently died with carcinoma involving the peritoneum or pleura yielded doubtful results. Of the remaining 130 specimens which did not show any evi-

dence of carcinoma at autopsy or surgery, one specimen, as stated previously, was misdiagnosed as carcinoma on cytologic examination.

Table 1 summarizes the findings.

REFERENCES

1. EHRLICH, P., quoted by Wihman.[5]
2. LÜCKE, A., AND KLEBS, E.: Beitrag zur Ovariotomie und zur Kenntnis der Abdominalgeschwülste. Arch f. path. Anat. u. Phys. u. f. klin. Med., **41:** 1–14, 1867.
3. QUINCKE, H.: Ueber fetthaltige Transudate, Hydrops chylosus und Hydrops adiposus. Deutsch. Arch. f. klin. Med., **16:** 121–139, 1875; Ueber die geformten Bestandtheile von Transsudaten. *Ibid.*, **30:** 580–588, 1882.
4. SCHMORL, G.: Die pathologisch-histologischen Untersuchungsmethoden. Ed. 10. Leipzig: F. C. W. Vogel., p. 67, 1918.
5. WIHMAN, G.: A contribution to the knowledge of the cellular content in exudates and transudates. Acta med. Scandinav., Suppl. 205, **130:** 1–124, 1948.

CYTOLOGIC DIAGNOSIS OF CARCINOMA OF THE PROSTATE*

PETER A. HERBUT, M.D.

From the Department of Pathology, Jefferson Medical College, Philadelphia, Pennsylvania

The examination of prostatic secretions for neoplastic cells is made in an attempt to arrive at an earlier diagnosis of carcinoma of the prostate. In a previous preliminary communication, Lubin and I[1] reported 17 cases of carcinoma of the prostate encountered in 100 consecutive patients. Since the method is simple and the results appeared promising, we made a further investigation and wish to summarize our recent findings, as of September 1, 1948.

METHOD

Secretions are secured by digitally massaging the prostate. Care should be taken to stroke as nearly as possible only that portion of the organ which contains the suspected tumor mass. Unless the tumor extends into the seminal vesicles, the latter should, if possible be avoided since their contents serve merely to dilute the desired prostatic secretions. Although secretions may be collected in a test tube and then transferred to glass slides, we have found it more desirable to deposit the fluid directly upon the slide and prepare the smears immediately. There are two reasons for this. First, sometimes the secretions are scanty and are easily dissipated on the walls of a test tube, leaving little or nothing that can be transferred to the slides. Second, it is desirable to fix the cells immediately to prevent autolytic changes. Transporting them to the laboratory in a test tube permits both autolysis and evaporation. Accordingly, as the drops of secretion appear at the external urethral meatus, one drop is collected on each of three clean, new, glass slides. Each drop is then covered with another clean slide and compressed. This allows the fluid to spread evenly over the surfaces. In order to insure a thin film of uniform thickness, the top slide is then pulled over the bottom one several times. The ultimate smear should not be any thicker than that obtained in performing an ordinary erythrocytic count. When the desired thickness is obtained the slides are pulled apart and immediately, while still wet, dropped into a mixture of equal parts of 95 per cent alcohol and ether, in which they are allowed to fix for from 15 to 20 minutes. They are then stained by the ordinary Papanicolaou technic.[2] We have arbitrarily chosen and routinely make six smears from each case. If, for some reason, staining cannot be carried out immediately, or if one desires to send the slides by mail, one need only remove the smears after fixing them for the required time and allow them to dry at room temperature. We have stained smears one month after fixation and have noted no appreciable difference in staining characteristics. In examining the preparations every portion of every slide is scanned under a magnification of approximately 100 times and, when atypical cells are encountered, under a magnification of 400 times. We have not found it necessary to use oil immersion.

In order for one to familiarize himself with other than neoplastic cells, he cannot do better than examine secretions from normal, inflamed, and hypertrophied prostates. In addition, it is most desirable to examine epithelial cells from the urethra, prostate and seminal vesicles. Cells from the former location can be readily obtained from cadavers and cells from the latter two organs can be obtained from surgically removed specimens or at autopsy. Epithelium from the urethra or seminal vesicle is best secured by scraping the organ with a scalpel and smearing the juice. Prostatic tissue is sometimes dry and,

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, September 27, 1948.

therefore, we have found it most convenient to secure a portion of the tissue measuring about 5 mm. in diameter and to crush it thoroughly with a Kelly clamp. All the fibers and grossly visible pieces of tissue are then removed and only the juice is smeared. In some instances, it is necessary to add a drop of saline and crush the tissue within the saline in order to secure any secretion. In any case, the smears of the scrapings and of the crushed tissue are treated in the same way as are those of the prostatic secretions. Likewise, in order for one to familiarize himself with the appearance of neoplastic cells, he may study cancerous prostatic tissue, obtained surgically or at necropsy, which is treated in a similar manner.

MACROSCOPIC APPEARANCE OF SECRETIONS

Grossly there is no feature that enables one to distinguish secretions of cancerous from noncancerous prostates. The normal amount of secretion, 1 to 3 cc., is obtainable from each type of prostate, and a decreased amount does not necessarily mean that the patient has carcinoma. The color, too, in each group is the same. In some patients, however, with advanced carcinoma in which the entire organ is transformed into a stony hard mass, one may be able to obtain only a few drops of thin, watery material, or there may be no material available. Likewise, in patients who have had a previous transurethral resection, whether the prostate is neoplastic or not, it may be impossible to secure any fluid.

MICROSCOPIC APPEARANCE OF SECRETIONS

Non-neoplastic cells. There are two types of cells that may be found in noncancerous prostatic secretions, namely, epithelial and inflammatory. Epithelial cells are of three types: urethral, prostatic, and seminal vesicular.

Urethral cells are easy to identify. They are the same large, flat, pavement cells that are so frequently encountered in urinary sediment. They are polyhedral, round, oval or irregular in shape, and always have sharp borders. They contain an abundant amount of homogeneous cytoplasm that normally takes the green stain but on occasions appears orange, yellow, blue, or has various shades of these colors. The nuclei are always round, oval, small, and central or eccentric in position. They are evenly and, as a rule, deeply stained.

Normal *prostatic cells* usually appear singly or in small groups of not more than three or four cells. Each cell is relatively small, measuring not more than 10 to 15 microns in diameter. Single cells are usually cuboidal, oblong, or somewhat irregular in shape, and their borders are always sharp. The cytoplasm is moderate in amount, homogeneous, and usually stains a light green or, at the most, bluish green. The nucleus occupies about one-half of the cell volume, is round or oval and is of uniform size in different cells. Its borders are sharp and its chromatin is usually clumped into minute granules. There are no nucleoli. The over-all staining is light or moderately dark blue. When found in small groups, normal cells are either irregularly arranged or they appear in linear apposition. Sometimes single cells show considerable degeneration, in which case they are two to four times the normal size, their borders usually rounded but distinct, their cytoplasm foamy, reticulated or definitely vacuolated, and their nuclei pushed aside, rounded or sometimes elongated. Secretions from hypertrophied prostates frequently reveal large, irregular masses of epithelial cells, or small, rounded, circumscribed nuggets. In the former, the cells are

essentially similar to those found in normal prostates, except that they are considerably larger and that their borders are almost always polyhedral. They can be readily distinguished from neoplastic cells, however, because their margins are always distinct. The clumps that appear in nugget form are usually quite small and, as a rule, consist of not more than half a dozen cells. The circumference of the group is usually sharply defined, but the cytoplasm forms one syncytial mass and shows no cellular boundaries. The nuclei are relatively small, round or oval, and not numerous. They are similar to those already described. The regularity and small size of these nuclei, together with their paucity of numbers readily distinguishes them from somewhat similar groups that are sometimes found in cancerous prostates.

Epithelium from *seminal vesicles* is quite characteristic. The cells are almost always single and are two to four times the size of prostatic cells. They are almost always round, have sharp borders, disclose round, deeply staining, eccentric nuclei, contain abundant deep bluish green cytoplasm, and frequently reveal cytoplasmic brown granules. In addition, spermatozoa are almost always found in great numbers throughout the smears and are agglutinated to the cells.

Inflammatory cells are recognized as a rule without difficulty, for they are of usual appearance. Neutrophils, lymphocytes, plasma cells and macrophages may be present.

Neoplastic cells. Neoplastic cells that are easiest to identify are those which appear in large, irregular sheets (Fig. 1). The borders of the masses are irregular, frayed, and indistinct. The over-all hue varies from bluish green to light brown or very light orange. The individual cells are usually about twice the normal size, or they may equal those from hypertrophied prostates. The cell boundaries, however, are always indistinct or, as a rule, completely absent, so that the cytoplasm forms a large syncytial mass. Close inspection under a magnification of 400 times discloses it to be bluish green, light brown, light orange or pink, and to be reticulated or finely granular. The nuclei are usually quite numerous so that they frequently overlap. They occupy about one-half or more of the cell volume and are round, oval, elongated, or slightly irregular. Their borders are sharply defined but are not rimmed by a conspicuous membrane. Staining is homogeneously light or dark blue but never black. The chromatin is evenly dispersed throughout the cell although, occasionally, smudgy, ill defined, coalescing clumps may be visualized. There is, however, no distinct chromatin grouping to form sharp granules such as are frequently seen in normal cells. Infrequently the nucleus may contain one or more distinct nucleoli. At other times, masses of epithelial cells are essentially similar, but the nuclei show an extreme variability in shape, size, and staining qualities (Fig. 2). They are round, oval, or irregular. Some are three or four times the normal size, while others are small and extremely hyperchromatic. Occasionally there are groups of cells that disclose irregular scalloped borders, distinct round margins, abundant vacuolated or reticulated cytoplasm, and deeply stained round or oval nuclei. Similar scalloped clumps occasionally reveal abundant, more or less homogeneous, light bluish green or gray cytoplasm with relatively small, ir-

regular, hyperchromatic nuclei. Finally, clumps of neoplastic cells may appear in rounded or oblong nuggets (Fig. 3). They are usually quite large and may occupy as much as one-third, or even one-half, of a microscopic field under a magnification of 400 times. The outline of the mass is always sharp. The cytoplasm forms a solid syncytial mass that stains pink or bluish gray. The cytoplasmic mass shows no cellular borders and is homogeneous, slightly vacuolated or reticulated. The nuclei are quite numerous, frequently overlap, and are evenly distributed throughout the clump. They are one and one-half to two times the normal size, are round, oval, or oblong, stain light or dark blue and are finely reticulated but show no sharp clumping of the chromatin. They may or may not contain nucleoli.

Single neoplastic cells vary in size from that of normal to that of the cells found in clumps just described (Fig. 4). The cell borders are always indistinct and irregular. The cytoplasm is scanty or imperceptible or is more abundant, stains bluish green or light gray and is drawn out into irregular fading processes. The nuclei are similar to those in the neoplastic cells already described. They may be large or small, lightly or deeply stained, and may or may not contain nucleoli.

RESULTS

Up to September 1, 1948, a total of 520 preparations from a total of approximately 480 patients were examined. In this group there was a total of 66 patients with carcinoma of the prostate, in whom the diagnosis was proved by paraffin sections in 48 and was certain clinically in the remaining 18. Neoplastic cells were found in secretions of 54 of the 66 patients (81.8 per cent). An equivocal diagnosis of cancer was rendered in 16 patients, but in most of these patients the questionable diagnosis was rendered during the first year of our project, and a recent review of the smears revealed that the suspicion in most instances was not justified. Also in the early part of the work there were three false diagnoses of cancer. With the experience which we have now accumulated, we believe that these mistakes would not be repeated. At present, when the clinical diagnosis does not coincide with the cytologic impression, the study is repeated at least once again. Also, if irregular cells are encountered that are not typical of carcinoma one or more re-examinations are performed after which the smears are called positive or negative.

SUMMARY

Examination of prostatic secretions for neoplastic cells is of value in the diagnosis of carcinoma of the prostate. A positive cytologic diagnosis has its greatest merit in cases that are clinically uncertain. A negative smear, on the other hand, does not mean that the patient is free of cancer. The method is recommended as a routine diagnostic laboratory procedure.

REFERENCES

1. Herbut, Peter A., and Lubin, E. M.: Cancer cells in prostatic secretions. J. Urol., 57: 542–551, 1947.
2. Papanicolaou, G. N.: A new procedure for staining vaginal smears. Science, 95: 438–439, 1942.

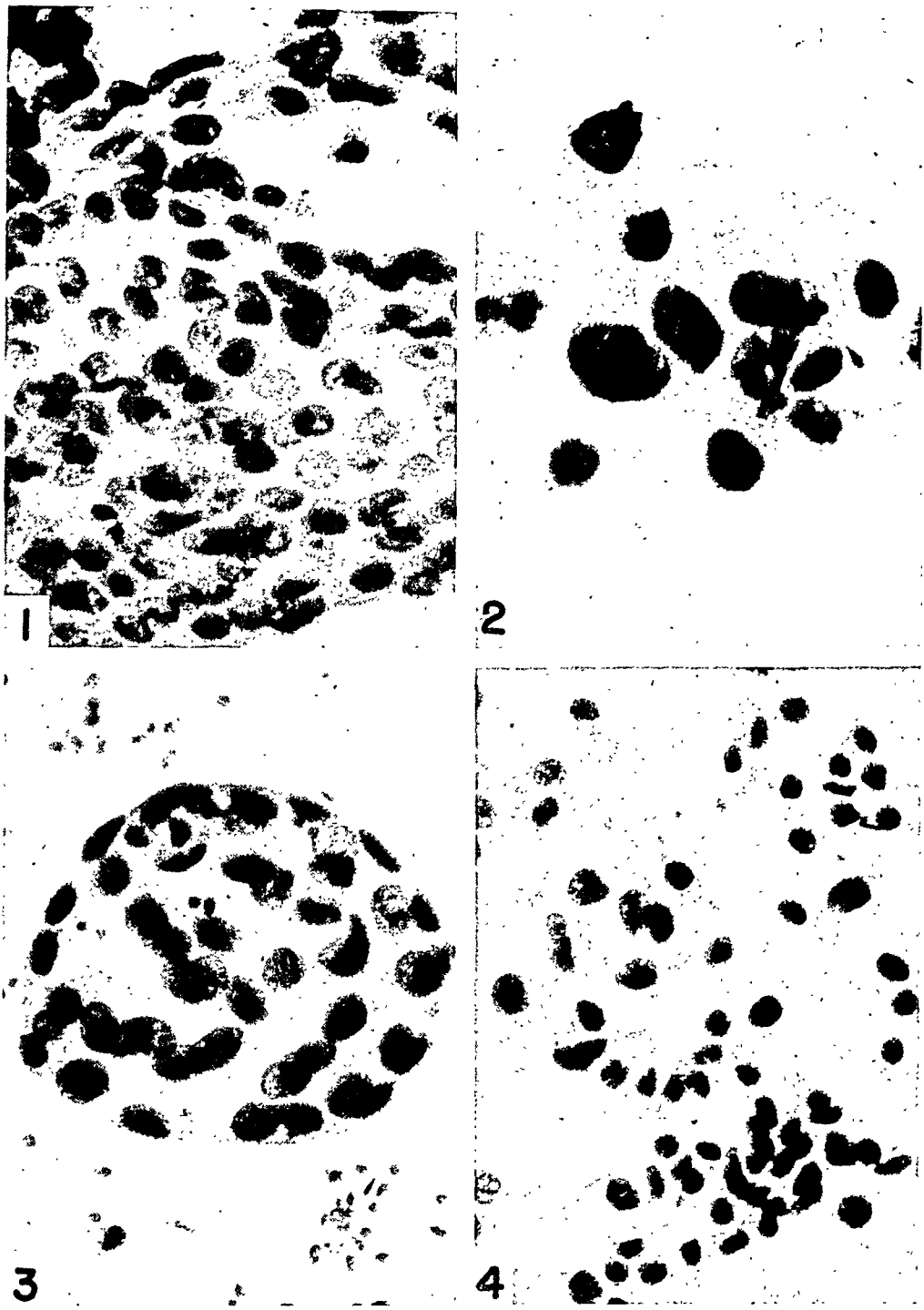


FIG. 1. A sheet of malignant neoplastic cells showing relatively large nuclei and coalescing, ill-defined cytoplasm. × 400.

FIG. 2. A clump of cancer cells showing considerable hyperchromatism and irregularity. × 400.

FIG. 3. A circumscribed nest of malignant neoplastic cells with cytoplasm forming solid masses and nuclei irregularly distributed. × 400.

FIG. 4. Single neoplastic cells showing scanty or imperceptible cytoplasm. × 400.

TUMOR CELLS IN BRONCHIAL SECRETIONS

An Experimental and Statistical Study*

MAX APPEL, M.D., and THEODORE T. BRONK, M.D.

From the Burnham City Hospital, Champaign, Illinois, and the Department of Pathology,† Michael Reese Hospital, Chicago, Illinois

It is generally agreed that results obtained in the treatment of carcinoma of the lung are unsatisfactory. These poor results are due in large measure to the fact that significant signs and symptoms of pulmonary malignancy do not become evident until the disease is far advanced. The diagnostic value of roentgenography is limited by the fact that it is frequently difficult to distinguish neoplastic from inflammatory lesions of the lung. Bronchoscopy is also of limited diagnostic value because the great proportion of bronchogenic tumors are beyond the reach of the bronchoscope, and also because facilities for bronchoscopy are not available in most smaller communities. For these reasons any diagnostic procedure which will enable the pathologist to make an early diagnosis of carcinoma of the lung is worthy of complete consideration.

The study of exfoliative cytology has provided the pathologist with such a weapon. The value of this diagnostic method has already been established.[2-5] Recently we had the opportunity to observe and study a case which illustrated in striking fashion the superior accuracy of this diagnostic technic over previously employed methods. The patient was a 58 year old white woman who was admitted to the hospital complaining of paroxysmal attacks of dyspnea associated with some cough and weight loss. Physical examination revealed absent breath sounds in the region of the angle of the scapula on the right side. Repeated radiographic examinations of the chest revealed, at times, areas of atelectasis on the right side and, on other occasions, well-aerated lung tissue, but at no time was any evidence of neoplasm detected. Bronchoscopy showed no evidence of tumor. Bronchial secretions were smeared and stained by the Papanicolaou technic. Clusters of tumor cells were seen (Fig. 1) in secretions obtained from the right main bronchus. Because of the finding of these tumor cells, and despite the absence of bronchoscopic evidence of tumor, and because the clinical signs were localized to the right lung, right pneumonectomy was done. Preliminary examination of the excised lung at first failed to reveal any evidence of tumor. Only after a careful dissection of the bronchial tree was a small area of thickening noted occluding a tertiary division of the right bronchus (Fig. 2). Microscopic sections of this area showed papillary projections of a primary carcinoma arising in the bronchial mucosa (Fig. 3). Despite the small size of the primary tumor, there was sufficient exfoliation of tumor cells to make them readily recognizable in the bronchial secretions before there was any definite clinical, radiographic, or bronchoscopic evidence of tumor.

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

This work was aided by a grant from the Ira Frank Fund.

Received for publication, January 17, 1949.

† This department is in part supported by the Michael Reese Research Foundation.

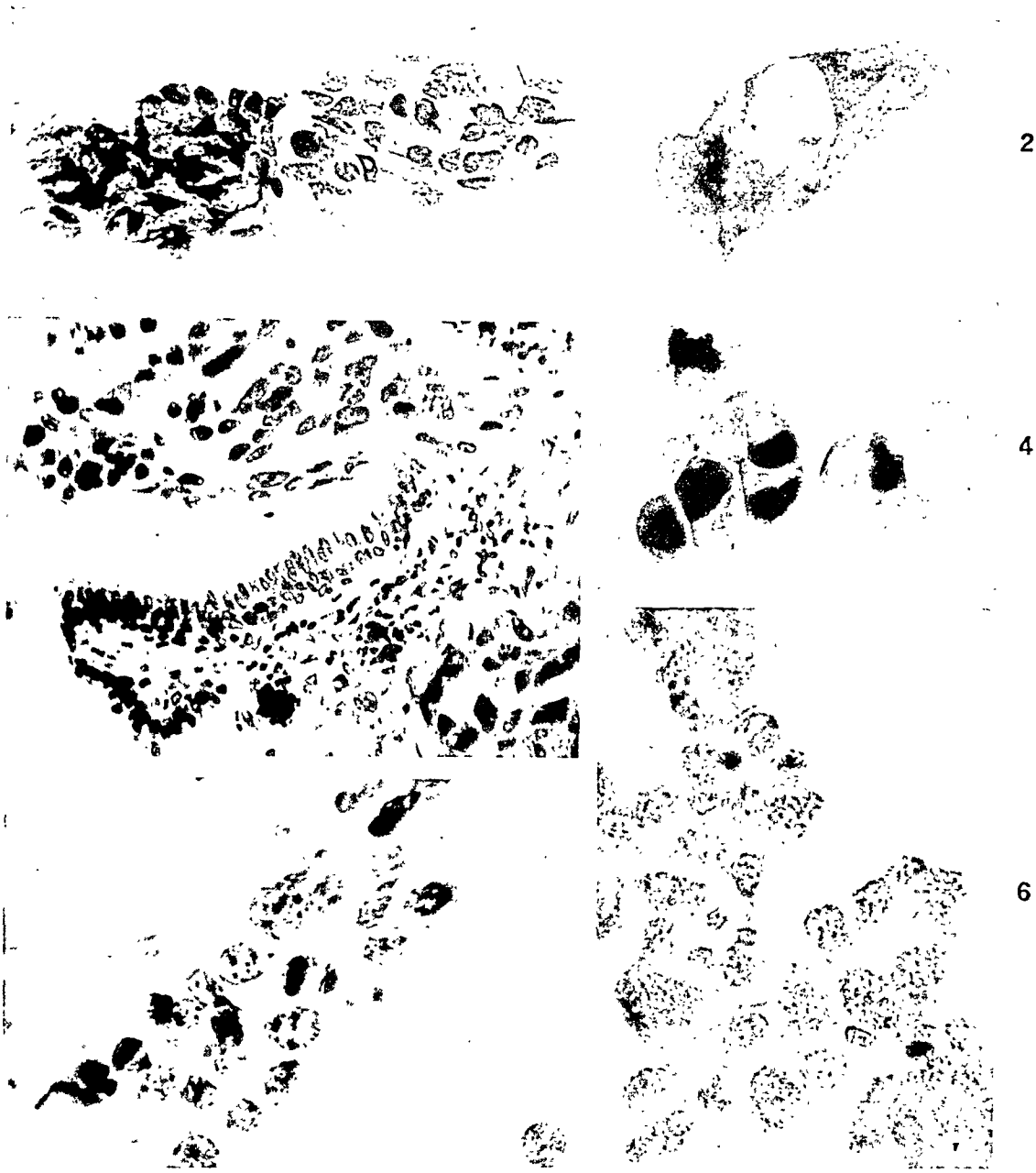


FIG. 1. Clusters of tumor cells found in bronchial secretions from patient with early bronchogenic carcinoma. Papanicolaou's stain. × 500.

FIG. 2. Early bronchogenic carcinoma found in tertiary division of right bronchus. Actual size.

FIG. 3. Photomicrograph showing papillary projections of tumor arising in mucosa of bronchus. Hematoxylin and eosin stain. × 500.

FIGS. 4–6. Tumor cells found in bronchial secretions of rabbits with transplanted pulmonary carcinoma. Papanicolaou's stain. × 900.

Because of this experience, it occurred to us that it might be of interest to conduct an experimental study with a view to determining (1) how soon after

the establishment of tumor growth, tumor cells can be identified in bronchial secretions, and (2) the proportion of cases of experimentally produced pulmonary neoplasms in which positive smears could be obtained. For this study we used the Brown-Pearce carcinoma, a readily transplantable tumor of the testis of the rabbit. The technic of transplanting the tumor into the bronchus has been described.[1] Attempts were made to obtain bronchial secretions by irrigating the bronchial tree with normal saline. Considerable technical difficulty was encountered. The animals did not tolerate the procedure well, many of them showing signs of asphyxia, and difficulty was encountered in recovering the saline. However, several successful aspirations were accomplished and tumor cells could be demonstrated by this technic (Fig. 4).

Because of the technical difficulty described above, this procedure was abandoned and attempts were then made to obtain bronchial secretions by the use of a swab. The swab was introduced into the trachea through a child's laryngoscope, gently rotated in contact with the tracheal mucosa, and the material obtained, smeared and stained by the Papanicolaou technic. The animals tolerated this procedure well and the results were gratifying (Figs. 5 and 6).

Smears were taken every third day, beginning on the sixth day following transplantation. In 17 of the animals, or 58 per cent, positive smears were found on the sixth day. By the end of the third week, 100 per cent of the animals showed positive smears. It must be emphasized that, in some cases, as many as 10 to 12 smears had to be taken before tumor cells could be identified in the bronchial secretions but at one stage or other in the development of the tumor-cells could be identified in all cases when sufficient smears were taken.

In order to correlate the appearance of tumor cells in the bronchial secretions with the appearance of grossly recognizable tumor in the lung, the animals were sacrificed as soon as positive bronchial smears were obtained. In none of the 17 animals showing tumor cells in the bronchial secretions at six days could tumor be recognized grossly. At this stage small microscopic nodules of tumor could be seen, but no gross evidence of tumor was found. The earliest time at which tumor could be recognized grossly was 10 or 12 days following transplantation.

In summary, it may be stated that at one time or other in the development of experimentally produced pulmonary carcinoma in rabbits, exfoliated cells can be found in the bronchial secretions in 100 per cent of cases. In 58 per cent of cases, tumor cells could be found in the bronchial secretions before the tumor was grossly recognizable and at a stage at which neither x-ray examination nor bronchoscopy could have identified the growth.

REFERENCES

1. Bronk, Theodore T., and Appel, Max: Intratracheal transplantation with the Brown-Pearce carcinoma. Cancer Research, in press.
2. Diggs, L. W.: Use of Wright's stain in diagnosis of malignant cells in bronchial aspirations. Am. J. Clin. Path., **18**: 293–302, 1948.
3. Herbut, P. A., and Clerf, L. H.: Bronchogenic carcinoma; diagnosis by cytologic study of bronchoscopically removed secretions. J. A. M. A., **130**: 1006–1012, 1946.
4. Papanicolaou, G. N.: Diagnostic value of exfoliated cells from cancerous tissues. J. A. M. A., **131**: 372–378, 1946.
5. Woolner, L. B., and McDonald, J. R.: Bronchogenic carcinoma; diagnosis by microscopic examination of sputum and bronchial secretions. Proc. Staff Meet. Mayo Clinic, **22**: 369–381, 1947.

A CYTOHISTOLOGIC STUDY OF BRONCHIAL SECRETIONS*

HOWARD L. RICHARDSON, M.D., WARREN C. HUNTER, M.D., WILLIAM S. CONKLIN, M.D., AND ARTHUR B. PETERSEN, M.D.†

From the Departments of Pathology and Surgery, University of Oregon Medical School, Portland, Oregon

The progress made during the past few years in the early diagnosis of bronchial carcinoma can be attributed to the study of secretional smears. However, there is little to be found in the American literature concerning the embedding and sectioning of material aspirated through the bronchoscope. In contrast, sputum has been cytohistologically studied by Boyd,[1] Zemansky,[4] and various European and South American workers mentioned in the monograph of Wandall.[3]

It is often difficult to obtain a good specimen from the bronchus for biopsy. In this study, a series of patients from each of two hospitals was treated separately in order to demonstrate the importance of (1) the experienced bronchoscopist in obtaining satisfactory material, and (2) the utilization of all aspirated material, for microscopic study.

METHOD

The first and larger series of cases is comprised of patients in a county charity hospital during the years 1946 and 1947, in whom a diagnosis of pulmonary neoplasm was considered. Because of inadequate follow-up records and lack of cooperation on the part of some patients, a number of cases of suspected carcinoma were omitted from this study. The diagnostic procedures on these patients were carried out by various members of the house staff with little standardization of technics. Many times part of the aspirated material was used for bacteriologic study.

The second series is composed of patients seen by one of us (W.S.C), a thoracic surgeon, during the years 1946 and 1947. All the procedures were standardized and directed by the same person. Complete data were available on all cases.

All of the material collected in the bronchoscopic trap was immediately fixed in formalin. At the pathologic laboratory the specimen was placed in a solution of saturated aqueous picric acid for four hours. It was next poured onto filter paper, the solidified material was packed into the cone, then treated like any other tissue and embedded in paraffin.

Routinely two sections were made, one from the surface and the other from the deeper structures of the block. The material was cut 6 to 8 microns in thickness and stained with hematoxylin, eosin and orange G. The orange G, especially, brings out the keratohyalin well. The sectioned material, which

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, September 24, 1948.

† Research Fellow in Pathology, Damon Runyon Fund.

represented a minute fragment of tissue was submitted for biopsy like any other tissue. The experienced pathologist can usually read such a slide in from three to five minutes.

TABLE 1

SERIES 1. EXAMINATION OF 119 COUNTY HOSPITAL PATIENTS FOR CARCINOMA OF LUNG. FREQUENCY OF POSITIVE FINDINGS BY VARIOUS METHODS OF EXAMINATION IN 17 PATIENTS WITH CARCINOMA OF LUNG. PROCEDURES NOT STANDARDIZED

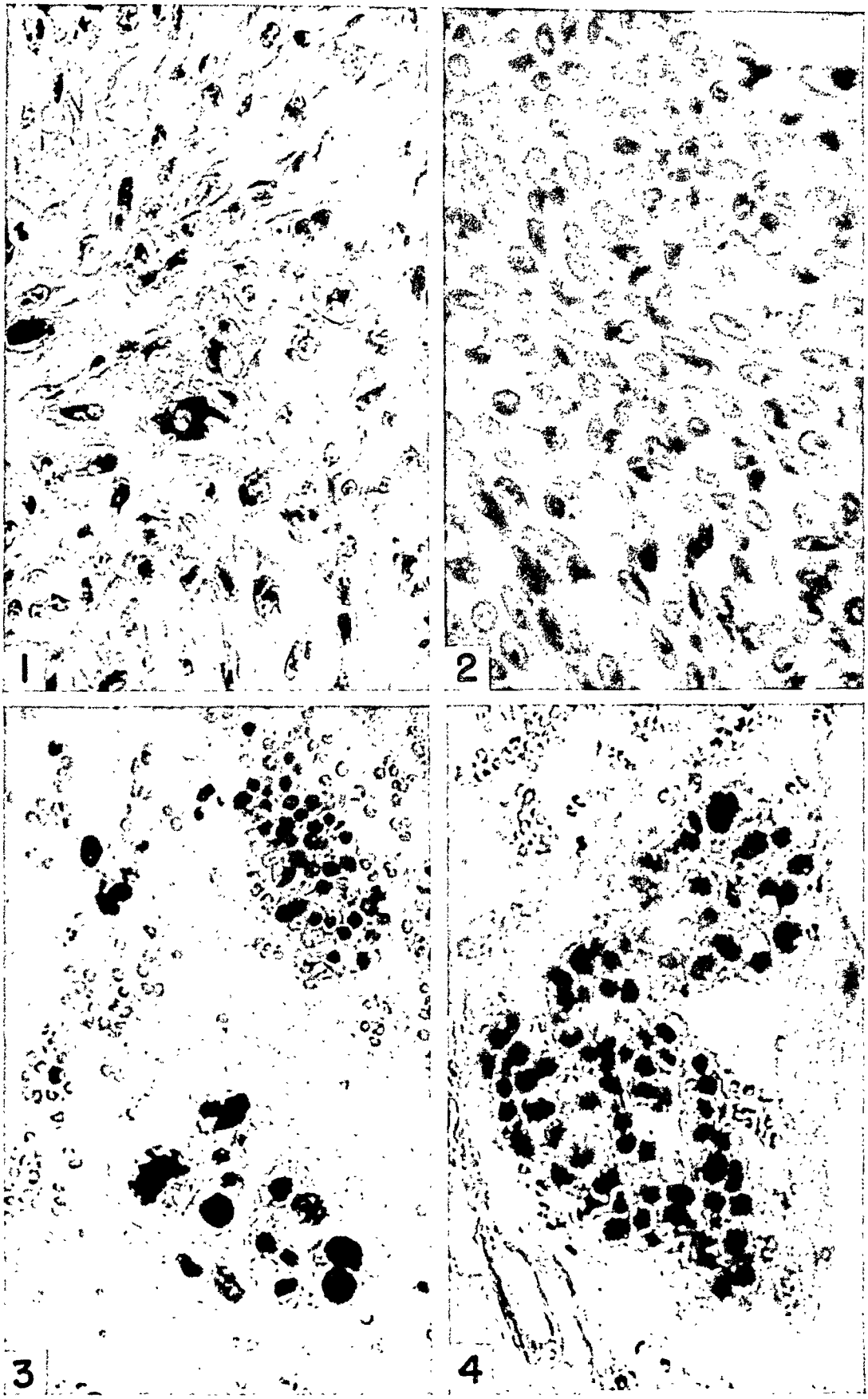
	NUMBER OF PATIENTS
Number of patients studied	119
Proved carcinoma of lung*	17
Carcinoma suspected by x-ray examination	17
Bronchoscopy performed	16
Tissue removed for biopsy	12
Carcinoma found on biopsy	11
Examination of material aspirated by bronchoscope	15
Carcinoma cells found	10 (66.7 per cent)
False-positive diagnosis	0
Examination of both sputum and aspirated material	7
Carcinoma diagnosed	3
In both sputum and aspirated material	3
In aspirated material only	2
In sputum only	1
In neither	1
Examination of sputum	9
Carcinoma diagnosed	5 (55.6 per cent)
Biopsy not obtained or negative, carcinoma cells found in aspirated material	1
Biopsy positive, no carcinoma cells found in aspirated material	4

* In 16 of these patients, a neoplasm was demonstrated at biopsy, surgery or necropsy; in one other patient typical "oat" type carcinoma cells were noted in aspirated material.

FINDINGS

Many normal epithelial cells were obtained as a result of scraping of the bronchial segment by the bronchoscope. Since most of the patients bron-

FIG. 1. Squamous cell carcinoma with bizarre keratohyalin formations.
FIG. 2. Immature cells from "oat cell" or "reserve cell" type of carcinoma from material aspirated from bronchus. × 600.
FIG. 3. Contrast the group of carcinoma cells below and normal cells above. × 600.
FIG. 4. Group of carcinoma cells in section of sputum. × 600.



FIGS. 1–4.

choscoped had some pathologic lung condition, monocytes were generally found. These cells are usually round, large, possess relatively small nuclei, and have abundant vacuolated cytoplasm containing phagocytosed carbon or hematin particulates. Masses of bacteria, basophilic debris, leukocytes, fibrin network with enmeshed red blood cells, mucus and protein, all may be present. Several surprising structures were found in aspirated masses of tissue, such as a fragment of pulmonary tissue from the wall of an abscess, a bronchial gland, anthracosilicotic fibrous nodules and giant cells in cases of tuberculosis. The

TABLE 2

SERIES 2. FINDINGS IN 48 PATIENTS IN WHOM STANDARDIZED STUDIES WERE MADE UNDER DIRECTION OF ONE BRONCHOSCOPIST

	NUMBER OF PATIENTS
Number of patients studied	48
Patients with carcinoma of lung proved at biopsy, surgery or necropsy	19
X-ray examination made	19
Carcinoma suspected on x-ray examination	19
Bronchoscopic examination made	19
Tissue taken at bronchoscopy for biopsy	15
Biopsy positive	13
Bronchial secretions aspirated	19
Positive findings in aspirated material	17 (89.4 per cent)
False-positive diagnosis of aspirated material	0
Patients in whom tissue was unobtainable or biopsy was negative, who had carcinoma cells in aspirated secretions	4
Patients in whom biopsy was positive and examination of aspirated secretion was negative	0

most difficult problem was to recognize epithelial metaplasia in cases of lung abscess and chronic inflammation where the hyperchromatism of the nuclei may be confusing.

Carcinoma of the lung did not produce any pathognomonic exudate. However, protein, fibrin, and clusters of lymphocytes made one suspicious. Each slide was scanned under the low power magnification and suspicious clumps of cells were studied further under high power.

Microscopically, the fragments of cancer tissue were classified as follows: (1) squamous cell carcinoma showing differentiation, (2) undifferentiated cell carcinoma including the so-called "oat-cell" carcinoma, and (3) adenocarcinoma. The most striking feature in keratinized squamous cell carcinoma (Fig. 1) was its cytoplasmic affinity for the orange G stain. Undifferentiated carcinoma cells tended to appear in small clusters. Characteristic were the scant cytoplasm, the large hyperchromatic nuclei, and the variable nucleoli (Figs. 2 and

3). Cells from adenocarcinoma, whether primary or metastatic, tended to occur in large clusters and were easily recognized as malignant because of the large cell size, basophilic staining qualities, multiple hyperchromatic nuclei, acinar formation, and variable secretion of mucus.

No concentrated effort was made in this series to study sputums. However, the results were surprising (Table 1, 55.6 per cent correct diagnoses) and warrant further cytohistologic investigation (Fig. 4). The sputum was collected over a 24 hour period by having the patient expectorate directly into a jar containing formalin, and the specimen was stirred to release entrapped air. The fixed material was then treated as previously outlined for bronchial aspiration.

In all, a total of 167 patients were studied and of these, 36 were diagnosed as having pulmonary neoplasm. Thirty-five of the 36 cases of pulmonary neoplasm, which have been designated as "proved cases", were verified on biopsy of excised bronchial tissue, during surgical intervention, or at necropsy. In the 1 other case, which is included in series number 1, typical "oat" type carcinoma cells were encountered in the bronchial aspiration. The results in the 2 series of cases are recorded in Tables 1 and 2. It is important to note in series 2 (Table 2), as contrasted to series 1 (Table 1), that in 4 patients in whom tissue was unobtainable or biopsy was negative the bronchial secretions contained carcinoma cells, and in no cases were the aspirations negative when the biopsy revealed neoplastic tissue.

SUMMARY

In the diagnosis of cancer of the lung, we have demonstrated the importance of immediate fixation of bronchial aspirations, of utilization of all material thus obtained, of standardization of technics, and of the services of an experienced bronchoscopist. We believe that the cytohistologic study of bronchial secretions is as satisfactory as, if not superior to, the smear technic. Furthermore, in embedding and sectioning, the groups of cells are neither torn apart nor superimposed, and the material is concentrated, thereby saving a great deal of time in the examination of slides. These are distinct advantages over smears.

Acknowledgments. Acknowledgments of assistance are made to the Oregon Division, American Cancer Society and its Diagnostic Smear Laboratory, Mae S. Kuge, technician. The study was aided by a Cancer Teaching Grant from the U. S. Public Health Service.

REFERENCES

1. Boyd, William: Discussion of paper by Herbut, P. A.: Cancer cells in bronchial secretions. Am. J. Path., **23:** 867–868, 1947.
2. Hunter, W. C., and Richardson, H. L.: Cytologic recognition of cancer in exfoliated material from various sources; useful modifications of the Papanicolaou technique. Surg., Gynec. and Obst., **85:** 275–280, 1947.
3. Wandall, H. H.: A study on neoplastic cells in sputum as a contribution to the diagnosis of primary lung cancer. Acta chir. Scandinav., Suppl. 93, **91:** 1944.
4. Zemansky, A. P., Jr.: Examinations of fluids for tumor cells. Am. J. M. Sc., **175:** 489–504, 1928.

CYTOHISTOLOGIC DIAGNOSIS OF MATERIAL ASPIRATED FROM STOMACH

Accuracy of Diagnosis of Cancer, Ulcer and Gastritis from Paraffin-Embedded Washings*

HOWARD L. RICHARDSON, M.D., FRANK B. QUEEN, M.D., AND FRED H. BISHOP, M.D.†

From the Department of Pathology, Medicine and Surgery, University of Oregon Medical School, Portland, Oregon

Attempts to discover methods and establish criteria by which single cancer cells could be reliably identified[6, 11, 12, 23] have been either impractical for routine diagnosis or unreliable, and pathologists have continued to rely upon patterns of growth in tissue rather than upon the appearance of individual cells for diagnosis.

The feasibility of the recognition of detached cells of uterine cancer in smears of vaginal fluid was demonstrated by Papanicolaou in 1928.[15] Papanicolaou and Traut[17] in 1943 showed the procedure to be highly reliable in a series of 3014 patients. Since then there has been a renewal of interest in cytologic methods of cancer diagnosis. Fluids from a variety of sources have been studied by an increasing number of investigators, using Papanicolaou's method or various modifications. Published work now indicates that the cytologic diagnosis is highly reliable in fluids from a variety of sources, equaling that of the biopsy in some.[6] To date, perhaps the greatest accuracy has been attained in the diagnosis of cancer of the uterus and cancer of the lung, with claims of accuracy from 96 to 99.7 per cent in uterine cancer,[13, 17] and of 82.4 per cent in pulmonary cancer.[8]

In April 1948 a conference of 61 pathologists and clinicians was called by the American Cancer Society for the purpose of evaluating the status of the cytologic method. It was concluded that the value of the cytologic method was established in some fields and that investigation of its usefulness in other fields should be continued.[3]

Since the early recognition of cancer of the stomach is one of the most difficult diagnostic problems, gastric fluids have been studied by several workers, chiefly with the object of detecting cancer. Cancer of the stomach is the most common of malignant diseases. It accounts for approximately 2 per cent of all deaths and 16.2 per cent of all cancer deaths in the United States.[14] It is also one of the least curable of cancers. Livingston and Pack[10] stated that definitive cures in unselected patients with gastric cancer are probably in the range of 2 per cent. Walters, Gray and Priestly[20] in 1942 reported 6.2 per cent five year cures in a series of 10,890 cases. Abrahamson and Hinton[1] considered only 5 per cent of

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, October 13, 1948.

† Fellow in Cancer Research, Damon Runyon Memorial Fund.

their 139 cases of gastric cancer candidates for a five year cure, and Welch and Allen[22] found that only 7 per cent of patients at the Massachusetts General Hospital survived at the end of five years. Wangensteen[21] stated that, "92 to 98 per cent of all patients who have gastric cancer will die of the disease within five years".

Cancer of the stomach is readily accessible to present day surgery. It possesses no characteristics or peculiarities rendering it inherently more malignant than other cancers. It is, however, one of the group of "hidden" or "cryptic" cancers in which signs or symptoms develop late, and for this reason, curability is distressingly low. The problem of gastric cancer is essentially one of

TABLE 1

RESULTS REPORTED BY OTHER AUTHORS IN THE CYTOLOGIC DIAGNOSIS OF CANCER FROM STUDY OF GASTRIC FLUID

	FRISHMAN AND GORIN[5] 1942	TOMENIUS[18, 19] 1947	PAPANICOLAOU AND COOPER[16] 1947	GRAHAM, ULFELDER AND GREEN[7] 1948	BLOCK AND BRYANT[2] 1948
Number of cases	70	72	137	50	—
Without cancer	25	67	110	26	—
With cancer	45	5	27	24	—
Cytologic diagnosis of cancer	20	0	10	15	—
Per cent positive in cancer cases	44.4	0	37	62.5	50-60
Number false positive	—	—	0	1	—
Per cent false positive	—	—	0	3.8	—
Number false negative	25	5	10	9	—
Per cent false negative	55.6	100	37	37.5	—

earlier detection of the lesion and any procedure which may promote earlier diagnosis deserves investigation. The results of some recent studies of gastric fluids with respect to the accuracy of cytologic diagnosis of cancer are shown in Table 1.

Frishman and Gorin[5] did not use Papanicolaou's method of examining stained smears of the alcohol-fixed sediment, but examined mostly unstained gastric sediment. Tomenius' study[19, 29] was not directed primarily at cancer, but was an attempt to determine the relative occurrence of cell types found in gastric juice or fluid aspirated from the normal stomach and in the more common pathologic conditions. After washing the stomach, he introduced buffered sodium bicarbonate solution, which was withdrawn in ten minute fractions through a special instrument containing four channels, through which secretions from the esophagus, stomach and duodenum could be separately obtained. The relative occurrence of 11 different types of cells was determined on air dried, alcohol

fixed, modified Giemsa-stained specimens of the sediments. His procedure was complicated and not suited for diagnostic purposes. However, his findings are of interest. There were no cells in about one-half of the fractions obtained from 46 cases. Leukocytes were found in 1.7 per cent of the fractions, and mucosal epithelial cells in 7 per cent. In 16 cases of chronic gastritis with persistent acid secretion the cellular picture was dominated by leukocytes in 70 per cent and eosinophils were present in 25 per cent. In 4 cases of cancer erythrocytes or leukocytes were abundant; in 1 case of cancer of the pylorus the cytology of the sediment was normal. In 7 cases of atrophic gastritis without acid secretion, 5 of gastric ulcer with gastritis, 10 of gastric ulcer without gastritis, and 3 miscellaneous conditions, the cytology could not be distinguished from that of the normal stomach. Only 2 lesions could be distinguished from the normal stomach: (1) chronic gastritis in which there was persistent acid secretion, and (2) cancer of the corpus and cardia only. (In these he did not make the diagnosis of cancer.)

Papanicolaou and Cooper's,[16] Graham, Ulfelder and Green's[7] and Block and Bryant's[2] studies were all directed primarily toward the diagnosis of gastric cancer. They recognized cancer when present in from 37 to 62.5 per cent of their cases.

The original purpose of our study was to determine the reliability of the cytologic diagnosis of gastric cancer, using the embedded section technic. It was soon obvious that the sections contained not only isolated cells but also frequently minute fragments of tissue, so that we were concerned not merely with cytology but also with histology. Our method of diagnosis might therefore, be called *cytohistologic*. As the work progressed, we believed it possible, with a fair degree of regularity, (1) to differentiate between a normal and a pathologic stomach, and (2) to recognize cancer, ulcer, and gastritis from the findings in the paraffin-embedded material obtained by washings from the fasting stomach. Therefore, our data are analyzed to determine the validity of these concepts, as well as to determine our diagnostic success in gastric cancer.

TECHNIC

Gastric washings are obtained by injecting and withdrawing several times 30 ml. of normal saline (1) following an Ewald meal, (2) following an alcohol-histamine meal, or (3) from the fasting empty stomach. The washings are added immediately to an equal volume of 10 per cent formalin. In the laboratory sufficient saturated aqueous picric acid solution is added to precipitate the protein and solidify the mucus. After fixing for four hours or more, the supernatant fluid is decanted and the residuum is passed through a porous filter paper (No. 202 Rapid, Reeve-Angel Co., New York, N. Y.). The precipitate is washed down to the tip of the filter paper if necessary, and is dehydrated, embedded and sectioned by the usual tissue technic. Two or three sections are cut from different levels within the block and stained with hematoxylin-orange G-eosin, using the following technic.[9]

1. Sections at 4–6 microns of tissue fixed on slides with egg albumin.
2. Xylol, 5 minutes.

3. Xylol, 5 minutes.
4. Absolute isopropyl alcohol, 3½ minutes.
5. Absolute isopropyl alcohol, 3 minutes.
6. 95 per cent isopropyl alcohol, 3 minutes.
7. 95 per cent isopropyl alcohol, 3 minutes.
8. Wash in running water until clear.
9. Hematoxylin (Ehrlich's), 3–8 minutes.
10. Wash in running water, 10–15 minutes.
11. Lithium carbonate (3 drops, saturated solution in 100 ml. water), 1 minute.
12. Wash in running water until clear.
13. 50 per cent isopropyl alcohol; rinse about 10 times.
14. 70 per cent isopropyl alcohol; rinse about 10 times.
15. 80 per cent isopropyl alcohol; rinse about 10 times.
16. 95 per cent ethyl alcohol; rinse about 10 times.
17. Orange G and eosin mixture, 3 minutes (Formula: 45 ml. eosin Y, 5 per cent in 95 per cent ethyl alcohol; 55 ml. orange G, 5 per cent in 95 per cent ethyl alcohol; 0.2 Gm. phosphotungstic acid).
18. 95 per cent ethyl alcohol; rinse about 10 times.
19. 95 per cent ethyl alcohol; rinse about 10 times.
20. Absolute isopropyl alcohol; rinse about 10 times.
21. Absolute isopropyl alcohol; rinse about 10 times.
22. Xylol, 3 changes, rinsing about 10 times in each.
23. Mount in Canada balsam.

Sections are examined with the low power objective, and suspicious areas are studied under the high power objective. This requires from three to five minutes' study per slide.

The greatest number of satisfactory specimens was obtained following the alcohol-histamine meal. Examinations following the Ewald meal were discontinued early in the investigation, as it was found that particulate debris interfered with satisfactory cellular interpretation. Immediate fixation is very important; otherwise, cells are digested within a short time and cellular interpretation is impossible.

The standard Papanicolaou stain does not give a satisfactory nuclear-cytoplasmic contrast in embedded material following formalin fixation, apparently because of the presence of precipitated formalin granules and because of changes in pH of the cell cytoplasm. Bismark brown and light green SF stain the cytoplasm of embedded cells so deeply that the nuclei are likely to be obscured. Elimination of these two components has produced a stain giving better contrast.

CYTOHISTOLOGY OF GASTRIC SECRETION AND CRITERIA FOR DIAGNOSIS

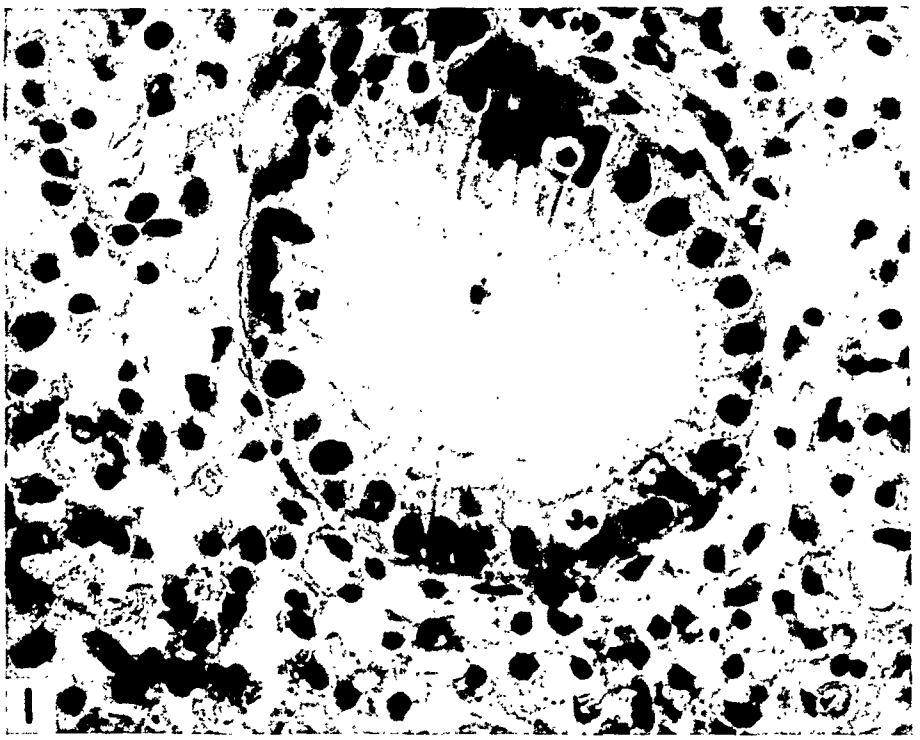
Normal stomach. The bulk of the cells are found to be of squamous type. Presumably, these are swallowed from the mouth, esophagus, and upper air passages. Basophilic staining mucus is abundant. The epithelial cells from the upper gastrointestinal and respiratory tracts are large, having clear cytoplasm and a small vesicular nucleus. Small, intact segments of gastric mucosa are often found. Gastric mucosa epithelial cells are cuboidal or columnar. The cytoplasm is clear, light pink, and may be vacuolated. The nucleus is small, eccentric, vesicular or dark. If cytolysis has occurred, only nuclei may remain, and these may appear clumped. Cells cut transversely seem to be composed

mainly of their nuclei but uniformity in size, shape, staining qualities, chromatin network and nucleoli differentiate these cells from carcinoma cells. Scattered granulocytes may be present. In specimens from the unwashed stomach or in those containing saliva that has been swallowed, mucus, masses of bacteria, bacteria-laden granulocytes, and monocytes containing anthracotic granules are also found in variable numbers.

Gastritis. In gastritis the inflammatory changes and edema permit large strands of gastric mucosa to become detached by washing and to appear in the embedded material. The more acute the inflammation and the greater the edema, the larger and more numerous are the detached mucosal masses. In acute gastritis, the diagnosis is based upon epithelial changes alone. The mucosal epithelium contains leukocytes within vacuoles, and clusters of leukocytes are often found in the glandular crypts (Fig. 1). Edema is conspicuous in the subepithelial interstitial tissue, although there may be no increase in inflammatory cells. In chronic gastritis, the diagnosis cannot be made from the inconstant epithelial changes, but depends upon the findings of lymphocytes, granulocytes, eosinophils and plasma cells in the subepithelial interstitial tissue. In atrophic gastritis, the characteristic change is the presence of large vacuolated goblet cells in the mucosa, as described by Faber[4] (Fig. 2). The interstitial reaction is the same as in chronic gastritis. The advantage of embedded and sectional material is that mucosal masses remain intact, this grouping being necessary for the recognition of the various forms of gastritis.

Ulcer. In ulcer a fibrin mass (Fig. 3) containing granulocytes, eosinophils, plasma cells, and sometimes hematin-laden monocytes is found. These fibrin masses are believed to have been washed from the ulcer crater. In healing ulcers, regenerating epithelium with hyperchromatic nuclei may be found within the fibrin mesh. These cells should not be confused with carcinoma cells. The fibrin mesh containing inflammatory cells, which we believe must be present to diagnose ulcer, is well preserved in embedded and sectioned material. It may readily be destroyed by the smear technic.

Cancer. Cancer cells are usually larger than normal cells, though they may vary considerably in size and shape. Their cell borders are usually indistinct. Cancer cells are characterized by large, hyperchromatic nuclei, and relatively scanty cytoplasm (Fig. 4). The cytoplasm is often vacuolated, particularly in adenocarcinoma, and gives a variable stain, depending upon the state of cell preservation. In well-preserved cells the cytoplasm stains acidophilic; in poorly preserved cells, more basophilic. The nucleus stands out, due to its deep staining peripheral chromatin granules. Sometimes it is ring-formed, and sometimes it is uniformly dark-stained. Nucleoli are usually prominent. Often cancer cells occur in clusters, their irregularity of size and shape, indefinite cell boundaries, and variability in nuclear size and chromatism of adjacent cells offering additional aid in diagnosis (Fig. 5). Ulcerated cancers may contain fibrin masses as found in benign ulcer cases. With ulcerated cancer, however, the embedded aspirated material often contains large amounts of necrotic debris, and plasma cells and eosinophils are more abundant than granulocytes. In non-obstructed cancer a large amount of eosinophilic staining protein is present. In



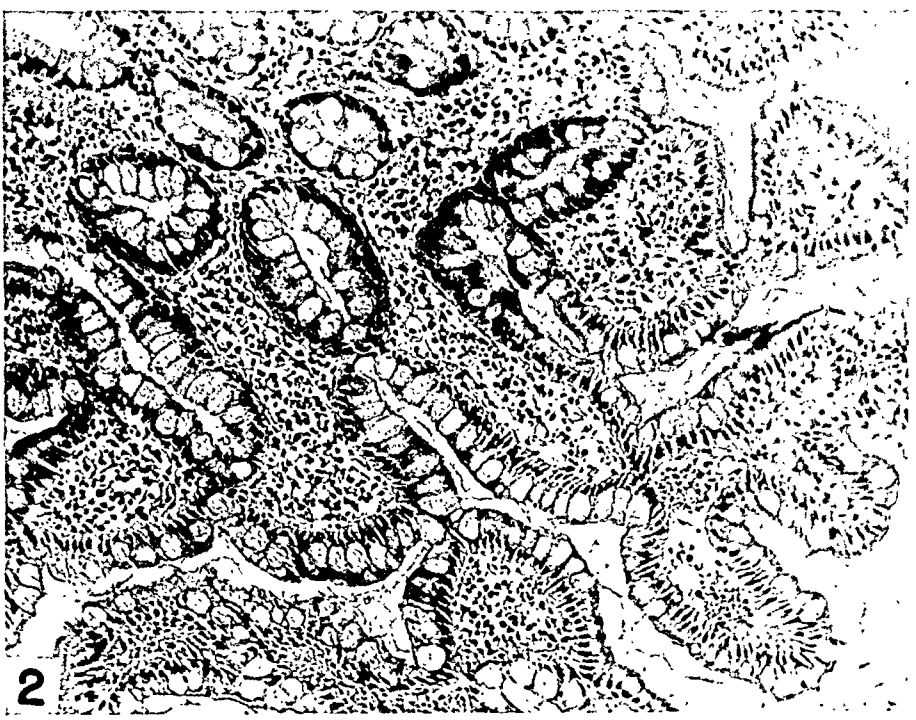


FIG. 1. Acute gastritis. Intraepithelial cytoplasmic vacuoles containing leukocytes characterize acute gastritis. × 500.

FIG. 2. Atrophic gastritis. Numerous epithelial goblet cells characterize atrophic gastritis. × 500.

obstructive cases basophilic protein is predominant. The presence of large amounts of protein is sufficient to characterize the stomach as pathologic and warrants repeating the examination.

Our criteria for the various cytohistologic diagnoses are summarized as follows:

Normal.

1. Squamous epithelial or normal gastric mucosal cells.
2. Few leukocytes.
3. Fibrin, erythrocytes and protein absent or very scanty.

Gastritis.

1. Many intracytoplasmic granulocytes in mucosal epithelium (acute gastritis).
2. Abundant leukocytes in subepithelial stroma, intracytoplasmic granulocytes inconstant (chronic gastritis).
3. Numerous goblet cells in mucosal epithelium (atrophic gastritis).

Ulcer.

1. Fibrin mass containing enmeshed leukocytes.

Carcinoma.

1. Clusters of enlarged, irregular cells with hyperchromatic nuclei and prominent nucleoli.

Numerous erythrocytes, granulocytes, protein, abundant fibrin, or large amounts of necrotic cell debris mean that a gastric lesion is present. Aspiration must be repeated to establish a definite diagnosis.

ANALYSIS OF RESULTS

To evaluate the usefulness of aspiration in the diagnosis of gastric lesion, the cytohistologic diagnosis must be compared with proved gastric lesions. Proved lesions were verified at surgery or autopsy; or by their clinical course, with positive x-ray and gastroscopic findings. Also, the cytohistologic diagnosis was established within a time sufficiently close to the clinical diagnosis that there was no doubt of the existence of the lesion at the time of the cytohistologic examination of the washings. For ulcer, this was in all cases within 20 days from the time of the clinical diagnosis; for gastritis, within nine days; for normal findings, within 20 days; and for cancer, within 3 months. In all of these conditions, the clinical manifestations remained unchanged during the specified periods.

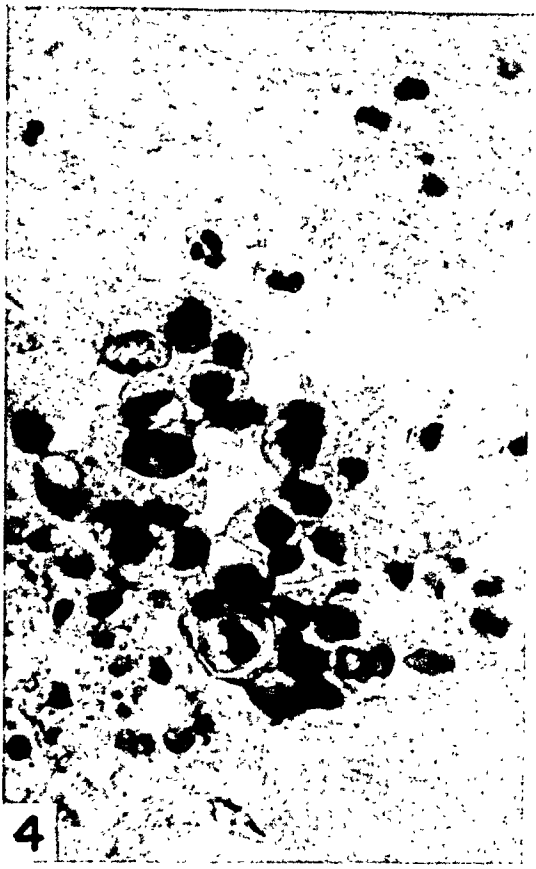
During the past two and one-half years we have examined a total of 297 aspirations from 235 patients. In 136 patients, the examination was considered as unsatisfactory because the clinical diagnoses were not established beyond doubt by the above-listed criteria. None of these 136 patients had gastric cancer. There were 99 patients with proved lesions. Of these, 14 had unsatisfactory cytohistologic examinations and in 7 the findings were discarded because

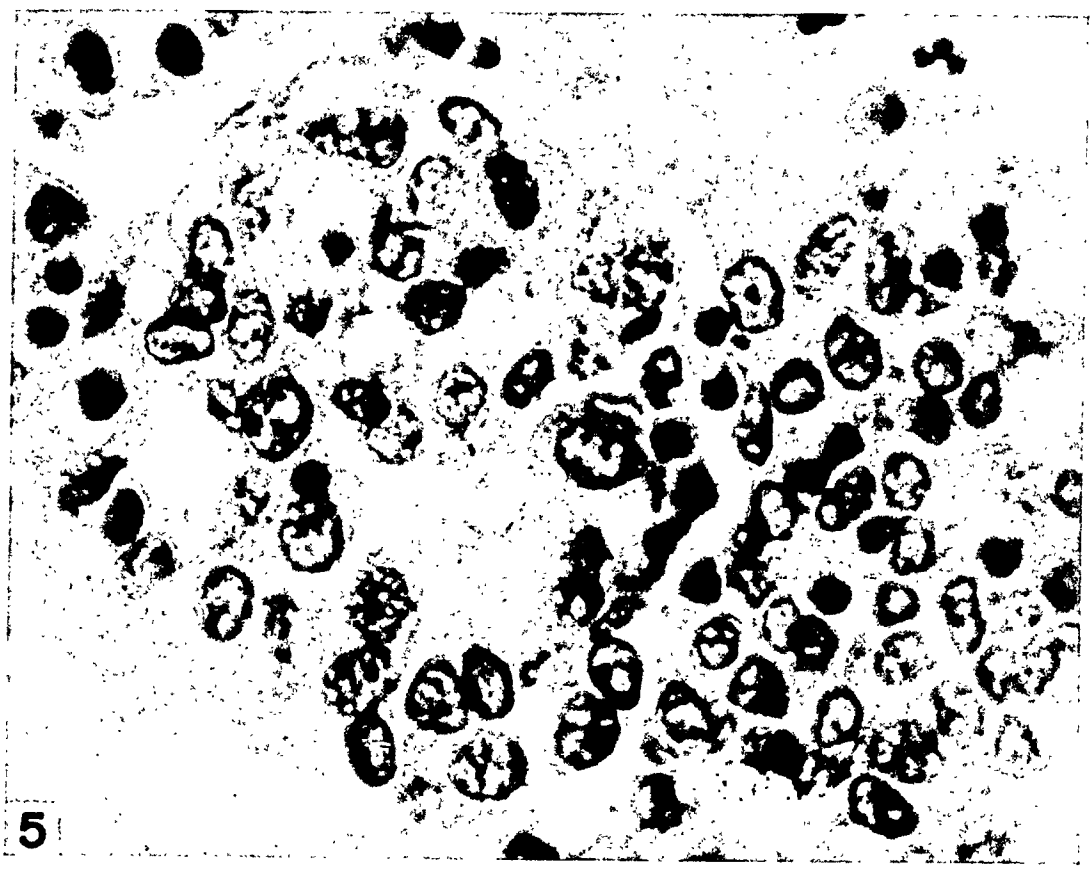
FIG. 3. Ulcer of the stomach. Ulcer is characterized by a fibrin mesh containing leukocytes. × 800.

FIG. 4. Adenocarcinoma of the stomach. Cancer cells are large, irregular, with hyperchromatic nuclei. × 640.

FIG. 5. Adenocarcinoma of the stomach. The nuclei of cancer cells are large and granular; chromatin is frequently peripheral and nucleoli prominent. The cytoplasm is relatively scanty. × 1000.







FIGS. 3-5.

the cytohistologic examinations were not performed within the established time limit. This leaves a total of 78 patients with proved gastric conditions to be discussed in this paper. Table 2 shows how these diagnoses were proved and classified:

A comparison of the cytohistologic diagnoses and the proved diagnosis in the 78 patients is shown in Table 3.

TABLE 2

DIAGNOSIS OF GASTRIC CONDITION AND METHOD OF PROOF IN 78 PATIENTS

DIAGNOSIS	NUMBER OF PATIENTS	PROVED BY	
		Operation or autopsy	Clinical course
Cancer	27	22	5
Ulcer	24	12	12
Gastritis	9	2	7
Normal	18	14	4
Totals	78	50	28

TABLE 3

CYTOHISTOLOGIC DIAGNOSIS COMPARED TO PROVED DIAGNOSIS IN 78 PATIENTS

PROVED DIAGNOSIS	NUMBER OF PATIENTS	CYTOHISTOLOGIC DIAGNOSIS									
		Cancer		Ulcer		Gastritis		Normal		"Pathologic"	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Cancer	27	13	48.1	10	37	3	11.1	0	0	1	3.8
Ulcer	24	1*	4.2	20	83.3	0	0	4	16.7	0	0
Gastritis	9	1*	11.1	1	11.1	6	66.7	2	22.2	0	0
Normal	18	0	0	2	11.1	3	16.7	11	61.1	2	11.1
Totals	78	15		33		12		17		3	

* In one patient, ulcer and cancer were diagnosed cytohistologically; in one patient, gastritis and cancer. Neither of these patients had cancer. Eighty cytohistologic diagnoses were made in these 78 patients.

Ulcer and gastritis were each diagnosed cytohistologically more often than they were actually present. We did not evaluate all errors, for our data were assembled with the primary purpose of determining our ability to make the diagnosis of cancer. The accuracy of cytohistologic diagnosis in each of the four proved conditions is shown in Table 4.

Cancer

In the 27 proved cases of cancer correct cytohistologic diagnosis was made in 13 (48.1 per cent). In more than one-half (14) the diagnosis was missed. In 5 patients, the diagnosis of cancer was missed because of inexperience, since in a later review, definitely recognizable cancer was found on the slides. In 3 instances, another specimen for examination was requested because of uncertainty

of diagnosis, but was not obtained. In the remaining 6 specimens, malignant cells were not present; 5 were called ulcer, and 1 gastritis. In all 6 of these patients, an ulcerated adenocarcinoma was present. In 4 of the 6 patients single aspirations were performed.

In no instance was diagnosis of normal stomach made among the patients with cancer, *i.e.*, all showed a pathologic stomach. We do not know whether cancer cells are always aspirated when cancer is present. Thirteen of our cases were positive on a single aspiration, while in one case 7 aspirations were necessary before cancer cells were found. In the 27 patients with cancer, there was

TABLE 4

ACCURACY OF CYTOHISTOLOGIC DIAGNOSIS OF GASTRIC CANCER, GASTRIC ULCER, GASTRITIS AND "NORMAL" SMEAR

PROVED DIAGNOSIS	NUMBER OF PATIENTS	CYTOHISTOLOGIC DIAGNOSIS			
		Proved correct		Proved incorrect	
		number	*per cent*	*number*	*per cent*
Cancer	27	13	48.1	14	51.9
No cancer	51	49	96.1	2*	3.9*
Ulcer	24	20	83.3	5**	20.8**
No ulcer	27	24	88.9	3	11.1
Gastritis	9	6	66.7	4†	44.4†
No gastritis	18	15	83.3	3	16.7
Normal	18	11	61.1	7	38.9
Not normal	60	54	90.	6	10.

* In the entire series of 221 specimens with satisfactory cytohistologic diagnosis, 194 showed no evidence of cancer. One of these was cytohistologically diagnosed cancer but was subsequently disproved on review of the slides. Thus, it may be said that there were 3 false-positive diagnoses of cancer in 194 specimens, an incidence of 1.6 per cent.

** In one patient diagnosed as having ulcer and cancer, ulcer was present but cancer was not.

† One patient with gastritis was diagnosed cytohistologically as having both cancer and gastritis; on review, the original diagnosis of cancer was seen to be erroneous.

a total of 42 aspirations of which 19 were positive. Papanicolaou believes that at least 3 examinations should be made in suspicious cases before arriving at a conclusion.[14]

There were 2 false-positive diagnoses of cancer in 51 patients, a false-positive error of 3.9 per cent. One was subsequently proved to be gastric ulcer, and 1 gastritis.

Ulcer

The accuracy of the cytohistologic diagnosis of ulcer in 61 proved cases, excluding the carcinoma cases, is shown in Table 4. Ulcer, when present, was correctly diagnosed in 20 of 24 patients or in 83.3 per cent. In the 20 correctly diagnosed ulcer cases, 32 aspirations were performed, 30 of which were positive

for ulcer. In the 5 patients (20.8 per cent) in whom the diagnosis was missed, 1 was diagnosed as having cancer as well as ulcer, and in 4 the stomach was believed to be normal. Only a single aspiration was examined in each of the 4 called normal. In the patient erroneously believed to have cancer, 2 subsequent examinations were diagnosed as ulcer. There were 3 false positive diagnoses in 27 patients (11.1 per cent) proved not to have ulcer. Two of these had no gastric lesion and 1 a gastritis.

Gastritis

The accuracy of the cytohistologic diagnosis of gastritis in 27 proved cases, excluding the cancer and ulcer cases, is also shown in Table 4. In 9 patients with gastritis the cytohistologic diagnosis was correct in 6 (66.7 per cent). Ten aspirations were performed in these 6 patients. In 4 patients (44.4 per cent) the diagnosis was missed; 1 was considered to have cancer as well as gastritis, 2 a normal stomach and 1 an ulcer. In these 4 patients only single aspirations were made. There were 3 false positive diagnoses of gastritis among 18 patients (16.7 per cent) proved not to have gastritis. These 3 patients had no gastric lesions.

Normal

The accuracy of the cytohistologic diagnosis of "normal smear" in 78 patients with proved gastric condition is shown in Table 4. In the 18 patients with no gastric lesions, 11 (61 per cent) were correctly diagnosed cytohistologically. Single aspirations were performed in these 11 patients. In 7 patients (39 per cent) the diagnosis was missed, 2 being recorded as "pathologic", 2 as ulcer, and 3 as gastritis. Requests were made for specimens for re-examination in the 2 instances reported as pathologic, but were not obtained. In 6 of the 7 patients, a single aspiration was performed, and in the other there were 2 aspirations. Six subjects were diagnosed normal among 60 proved not to be normal, constituting 10 per cent of false positive diagnoses. Four of these patients were proved to have ulcer and 2 gastritis.

While the accuracy (61 per cent) of the cytohistologic diagnosis was not high among subjects whose stomachs were normal, it is noteworthy that among all those with lesions, the diagnosis was accurate in 90 per cent of cases.

DISCUSSION

An attempt has been made to answer three questions in this study:

1. How accurate is the cytohistologic method of utilizing embedded and sectioned stomach washings for diagnosis of gastric cancer?
2. How accurately can gastric ulcer and gastritis be diagnosed by this method?
3. How accurately can normal stomachs be recognized by this method?

The recognition of a normal condition of the stomach was unimpressive. However, when the stomach was diseased, it was so recognized in 90 per cent of cases by the embedded section technic.

Ulcer can be fairly accurately recognized in embedded material. Gastritis is not so readily interpreted, probably because this diagnosis usually requires intact mucosal fragments.

The accuracy for the cytohistologic diagnosis of cancer was not great (48.1 per cent), which was in the range of that found by Papanicolaou and Cooper (37 per cent),[16] by Graham *et al.* (62.5 per cent),[7] and Block and Bryant (50–60 per cent).[2] On review of sections of the proved cases of cancer, 5 additional cancers were recognized that had been missed at the time of the original reports, which would have made a total positive diagnosis of 66.7 per cent instead of 48.1 per cent. All 5 were missed in the early period of the study and are not listed as positive among the cases here reported. This emphasizes the need of experience in interpretation.

All but one of the cancers in this series were large and advanced. However, this one was of particular interest. The cytohistologic diagnosis was reported positive before x-ray examinations or gastroscopy were performed. In the surgical specimen a small cancer approximately 1 mm. in diameter, invading the muscularis, was found in the margin of an ulcer. This indicates that even a minute cancer may be found by this method.

There are many sources of error in cytohistologic studies of gastric washings. The material obtained must be promptly fixed. It must, of course, contain cancer if it is to be recognized, just as with any tissue submitted for biopsy. Experience in interpretation is necessary, but it is not difficult for a trained pathologist to learn to differentiate the conditions commonly found.

The advantages of the embedded section technic are several. With embedded sectioned gastric sediments, there is a minimum distortion of cells, and the numerous mucosal fragments dislodged from lesions by the washing remain intact. By embedding and sectioning, the groups of cells are not separated as is likely to happen on smearing, thus enabling one to see a single layer of cells in normal architectural relationship, a most important advantage. With embedding and sectioning the sediment is concentrated so that more material can be examined in a shorter time. This is a distinct advantage for routine diagnosis.

SUMMARY

1. Material from 297 gastric aspirations obtained from 235 patients was embedded in paraffin and sectioned. Microscopic (cytohistologic) study was made of cells and fragments of tissue present. In 78 patients the diagnosis was proved at operation or autopsy, or by the clinical findings and course.

2. The procedure used, and the criteria for the cytohistologic diagnosis of cancer, ulcer, gastritis, and for the recognition of the normal smear have been presented.

3. Twenty-seven patients had proved cancer. Thirteen (48.1 per cent) were originally diagnosed cancer cytohistologically; on review of the sections, a total of 18 (66.7 per cent) were diagnosed as having cancer. A false-positive diagnosis of cancer was made in 3.9 per cent of patients.

4. Twenty-four patients had proved ulcers. Twenty (83.3 per cent) were microscopically diagnosed as having ulcer. A false-positive diagnosis of ulcer was made in 11.1 per cent of patients.

5. Nine patients had proved gastritis. Six (66.7 per cent) were diagnosed as

having gastritis. A false-positive diagnosis of gastritis was made in 16.7 per cent of patients.

6. Eighteen patients were proved to have no gastric lesions. Eleven (61.1 per cent) were diagnosed as normal. A false-positive diagnosis of normal was made in 10 per cent of subjects.

7. The embedded section (cytohistologic) technic presents certain advantages over the smear (cytologic) technic, important among which are the concentration of material and the better preservation of cellular relationships.

Acknowledgments are made to the Oregon Division of the American Cancer Society, Inc.; to its Diagnostic Cytological Laboratory, Mae S. Kuge, technician; to the U. S. Public Health Service for aid by a Cancer Teaching Grant; and to Moses E. Steinberg, M.D., for his cooperation in obtaining some of the gastric aspirations.

REFERENCES

1. Abrahamson, R. H., and Hinton, J. W.: Gastric carcinoma; comparative review of origin, diagnosis, and end-results in 583 patients. Surg., Gynec. and Obst., **84:** 481–490, 1947.
2. Block, M., and Bryant, H. C.: The diagnosis of gastric neoplasms by cytologic examination of gastric secretions. Abstract, Program of Scientific Assembly, 97th Annual Session American Medical Association, June, 1948.
3. Cancer News, Exfoliative Cytology. New York: American Cancer Society, Inc., 1948.
4. Faber, K.: Gastritis and Its Consequences. New York and London: Oxford University Press, p. 48, 1935.
5. Frishman, R. L., and Gorin, M. G.: Diagnostic significance of examination of gastric washings in patients suspected to have cancer. Klin. Med., **20:** 50–67, 1942.
6. Gates, O., and Warren, S.: A Handbook for the Diagnosis of Cancer of the Uterus by Use of Vaginal Smears. Cambridge: Harvard University Press, p. 9, 1947.
7. Graham, R. M., Ulfelder, H., and Green, T. H., Jr.: The cytologic method as an aid in the diagnosis of gastric carcinoma. Surg., Gynec. and Obst., **86:** 257–259, 1948.
8. Herbut, P. A., and Clerf, L. H.: Diagnosis of bronchogenic carcinoma by examination of bronchial secretions. Ann. Otol. Rhin. and Laryng., **55:** 646–655, 1946.
9. Hunter, W. C., and Richardson, H. L.: Cytological recognition of cancer in exfoliated material from various sources. Technical insert. Surg., Gynec. and Obst., **85:** 275–280, 1947.
10. Livingston, E. M., and Pack, G. T.: End-results in Treatment of Gastric Cancer. New York: Paul B. Hoeber, Inc., p. 48, 1939.
11. MacCarty, W. C.: Cytologic key to diagnosis and prognosis of neoplasms. J. Lab. and Clin. Med., **13:** 354–365, 1928.
12. MacCarty, W. C.: The malignant cell. J. Cancer Research, **13:** 167–172, 1929.
13. Meigs, J. V., Graham, R. M., Fremont-Smith, M., Janzen, Lt., and Nelson, C. B.: The value of the vaginal smear in the diagnosis of uterine cancer. Surg., Gynec. and Obst., **81:** 337–345, 1945.
14. Papanicolaou, G. N.: Diagnostic value of exfoliated cells from cancerous tissues. J.A.M.A., **131:** 372–378, 1946.
15. Papanicolaou, G. N.: New cancer diagnosis. Proceedings Third Race Betterment Conference, p. 258, 1928.
16. Papanicolaou, G. N., and Cooper, W. A.: The cytology of the gastric fluid in the diagnosis of carcinoma of the stomach. J. Nat. Cancer Inst., **7:** 357–359, 1947.
17. Papanicolaou, G. N., and Traut, H. F.: Diagnosis of Uterine Cancer by the Vaginal Smear. New York: The Commonwealth Fund, p. 44, 1943.
18. Tomenius, J.: The cytologic diagnosis of gastritis. Acta med. Scandinav., Suppl. Fasc. V, 1946.
19. Tomenius, J.: A study on gastric sediment. Acta med. Scandinav., Suppl. 179, p. 189, 1947.
20. Walters, W., Gray, H. K., and Priestly, J. T.: Carcinoma and Other Malignant Lesions of the Stomach. Philadelphia: W. B. Saunders Co., 576 pp., 1942.
21. Wangensteen, O. H.: The problem of gastric cancer. J.A.M.A., **134:** 1161–1169, 1947.
22. Welch, C. E., and Allen, A. A.: Carcinoma of the stomach. New England J. Med., **238:** 583–589, 1948.
23. Zemansky, A. P., Jr.: Examination of fluids for tumor cells; analysis of 113 cases checked against subsequent examination of tissue. Am. J. M. Sc., **175:** 489–504, 1928.

SYMPOSIUM ON THE CYTOLOGIC DIAGNOSIS OF CANCER

Discussion of Papers*

WILLIAM BOYD, M.D.

From the Department of Pathology and Bacteriology, University of Toronto, Toronto, Canada

Mr. President and members of the Society. Let me first thank you for the honor you have done me in asking me to be here. As I was traveling down in the train yesterday evening, I found myself asking what business I had to accept your invitation, because there was nobody less qualified to speak on the subject, certainly from the point of view of experience. I have no practical experience in exfoliative cytology. My own work and way of life since I have been in Toronto has gradually taken me away from surgical pathology and gynecology. But after all, the judge has no practical acquaintance with murder or rape, and when we appoint our judges we do not ask that they should have such experience of these affairs. I was comforted by this thought.

The papers of today's symposium have been particularly suitable for this audience, because they have shown us the technic and the technical difficulties, the pitfalls which the practical microscopist has to encounter. I was much interested in Dr. Saphir's historical backward look. It illustrated that there is nothing new under the sun, for he took us back a hundred years. So it is evident that exfoliative cytology did not originate with Dr. Papanicolaou, as he is the first to emphasize. But I venture to say, Mr. President, that if it had not been for Dr. Papanicolaou, there would not have been this symposium this morning. He has done a tremendous service both to the medical profession and to the public in his long work, which has eventually reached the clinician. No doubt there has been a good deal of pushing of the profession and of the pathologists in this matter. Take for instance these sentences quoted in the *New York Times* from the *Woman's Home Companion:* "The new method is inexpensive, painless, and at least 97 per cent accurate. If put into general use, it may almost wipe out this form of cancer." I am surprised that they put in "almost". When we get a statement like that it is no wonder that pressure has been brought to bear, and I think pressure is very good for us. If this pressure had not been applied it is probable that we would not have had this symposium.

I was particularly interested in Dr. Saphir's demonstration of the pseudoglandular structures, which might so readily lead to the wrong diagnosis. One of the most striking contributions, you will agree, was that of Dr. Appel. I must say I felt that if he could demonstrate cancer cells in my sputum, even though I felt in perfect health I would rush back to Toronto and have my lung out. The question is, which lung?

Listening to Dr. Richardson and seeing his beautiful preparations, I noted

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, Chicago, October 13, 1948.

Received for publication, December 9, 1948.

that he used the term exfoliative histology. He is really dealing with small biopsies with which many of us are more familiar than with the cytology of separated cells.

I was surprised to hear Dr. Herbut's remarkably encouraging results and to see his very conclusive demonstration.

There are a number of general subjects which I hope will be discussed. One of these is the question of carcinoma *in situ*. I would like to hear different views from different centers expressed. To my mind there is not the slightest doubt that there is such a thing. In fact, how could it be otherwise, as a cancer has to be *in situ* before it begins to invade deeper tissues. There is the possibility of a great abuse of this term. Statistics from different laboratories differ widely in regard to the incidence of carcinoma *in situ*. When the number of cases reported from any one laboratory is greater than the number of cases of cancer which might be expected on a statistical basis then we feel that there must be some misinterpretation of the lesions. The question is, are we not dealing with reversible changes? I feel that this is going to be one of the great contributions which this method is going to make in the future. By this means we can determine day by day, week by week, and month by month what is going on.

Dr. Papanicolaou has introduced this whole subject to the medical profession, and naturally attention has been focused on the examination of vaginal smears. We have also heard much today about the stomach, the prostate and the lung. Here is a field for future work, for these organs do not lie open to visual inspection or easy biopsy as does the cervix. One of the subjects which should be discussed is the matter of screening. When we analyze figures, particularly that 97 per cent which I have quoted, we must make it clear to ourselves and to the public that it all depends on which figures we are dealing with. Are we dealing with the screening of apparently healthy persons or are we dealing with the group of persons who come to the doctor because they think there is something the matter with them? Today it has been the latter group which we have been discussing but owing to public pressure we may very well be forced into considering the other group, and it is in the other group that the great difficulties occur, particularly the difficulty of the false negative. The patient comes to the doctor, has this simple test made, goes away, and the report comes back negative. Obviously it is not the skilled pathologist who is going to screen these hundreds and thousands of smears or sections. It is the technician. Only when the case is positive and still more when it is doubtful the expert looks at it, but when it is put down as negative the expert does not see it. The patient goes away and then if symptoms develop she says, "Well, thank God, it can't be cancer, because this great new test shows that I haven't got cancer." Of course, one of the great duties of the pathologist is to impress upon his medical confrères the extreme limitations of the method. The clinician would not think of saying when he gets a negative report for tubercle bacilli in the sputum that the patient has not got tuberculosis, especially from one examination.

Scientific discoveries which have been hailed on their first appearance as fresh

triumphs over nature lead to disillusion when their limitations are realized, but that is only if there is a tremendous ballyhoo started at the beginning about these discoveries. The function of the pathologist, and particularly of a society such as this, is to be most conservative in our outlook and to impress our clinical confrères with that conservativeness. The clinicians in turn, we hope, will impress the public, although that is a very difficult matter, because the people who have to be impressed are the gentlemen of the press and anybody who has tried to do that knows how hard that task is.

It is apparent from what we have heard today that the method has come to stay. At first there will be opponents and proponents. A number of persons have asked me this morning am I for or against it. What a question to ask! You do not ask the judge whether he is for or against. Of course I am for it. We must not be too enthusiastic; we must know what we are for. The general who is planning a battle has to count the costs in blood and to determine if his resources are adequate to the task ahead of him. He has to distinguish between the ideal battle and the real. We have got to do that too. I am talking about screening. The ideal, of course, is to have every woman examined once a year. No, because the day after she was examined she might start exfoliating cells. She ought to be examined every six months. No, surely you would not allow a tumor to grow for five and a half months! And what about the men; why should there be no ballyhoo about the men? After all, with the exception of the reproductive system, carcinoma is commoner in men than in women, and you never hear the press saying that men should be examined repeatedly.

A manufacturer of laboratory equipment has advertised the sale of materials for the Papanicolaou test together with a hundred slides of various types of cells found in cervical smears. One thing that the firm did not and could not sell was the most important ingredient, a competent pathologist. I leave it at that.

General Discussion

Dr. P. A. Herbut, Philadelphia, Pa.: Our experience with carcinoma of the lung is essentially similar to that of Dr. Richardson. In the preparation of slides, we use the same technic for carcinoma of the lung as we do for carcinoma of the prostate. Secretions are secured with the aid of a bronchoscope only from the area that drains the suspected site of the tumor. This is determined previously by x-ray studies of the chest. In cases where there is no x-ray shadow the bronchoscopist can usually tell where the lesion is by the source of the escaping secretion which is often hemorrhagic. Another point is that we would rather see cases in which there are no secretions whatsoever, because in these the lesion is recognized earlier, is presumably smaller, and the resectability is much greater. In such instances we use bronchial washings of that segment that contains the tumor. In washing the bronchus, however, one should introduce only that amount of saline which yields a sticky return. When too much saline is used the washings are watery and results are uniformly poor.

We have examined specimens from over 1000 different patients. In that group there were 237 proved cases of carcinoma of the lung. Smears of secretions were positive for neoplastic cells in 213 or almost 90 per cent. This includes all cases from the time we first started, when our positive results were around 70 per cent. The bronchoscopic biopsy was positive in this same group in only 86 cases or 36.2 per cent. One still hears, in certain places, that

a positive bronchoscopic biopsy should be obtained in 75 per cent of all pulmonary cancers. When this is so, I dare say that most of the lesions are late or that many peripheral tumors are never diagnosed correctly. In this same group of cases there was, however, indirect evidence of carcinoma in the form of stenosis, fixation, and deformity of the bronchus in an additional 46 patients. The cells were positive and bronchoscopic examination was completely negative in 75 cases, or in almost 33 per cent of the total.

We have several patients upon whom the surgeon operated, entirely on the basis of our positive cytologic diagnosis. When he opened the thorax he could see or feel nothing in the lung that would enable him to say the lesion was carcinoma. If we were not so positive in our diagnosis, he would not have removed the lung. Yet these cases are the earliest cases of carcinoma that I have ever seen. They showed involvement only of the mucosa and submucosa of the bronchus. On the other hand if one is not certain that the cells are neoplastic he should not make a dogmatic statement. The point is that one can be just as positive that a given lesion is carcinoma from a study of smears as one can be from a study of histologic sections.

Dr. M. G. Bohrod, Rochester, N. Y.: There is a general impression that pathologists as a class are not whole-heartedly in sympathy with the business of the cytologic diagnosis of cancer. If we could understand just why, and in what sense this is true, we might be able to avoid the dangers inherent in the use of the method and to take better advantage of its undoubted virtues. What the pathologist objects to is the excessive enthusiasm on the part not only of the lay public but also the general medical public. This tends to enhance the authority of the results of the cytologic method beyond scientific justification. It tends to overemphasize details of method into a ritual and demand needless conformity to a detail (a stain for instance) when a variety of others will do just as well. It tends to obscure the fact that there is no unique morphologic characteristic of a cancer cell, that the diagnosis is still one of probability and not a pathognomonic one. And above all, it tends to neglect older and well established methods, one of which, the biopsy, gives more information than the smear and is indeed the criterion on which the validity of the cytologic method is based.

Finally, there is the danger that over-indulgence in a method, making a fad of it, leads to the too-ready rejection of the method as soon as it becomes apparent that it cannot do everything that was hoped from it. The louder the ballyhoo that accompanies the introduction of a method the more likely this is to happen. The undoubted virtues of the cytologic method in cancer deserve to be retained and used in those situations in which they have their proper place. It is not necessary to puff them into a virtual answer to the problem of cancer diagnosis.

Why do we so often tend to go through this cycle of hyperenthusiasm—disappointment—neglect for methods of diagnosis or treatment? Why do we demand "belief" in a method? Here we deal with what may be called the praxiologic error. We ask a quasi-legislative function of our "tests", an accepted way of doing (praxis) that can replace complicated evaluation. So long as the test is "believed in" we can look to it for justification even when it leads us to a wrong conclusion. When we have had some disappointment we no longer "believe in" it, we repeal the legislation, and we refuse to use the method even for what it *can* do. Since pathologists are so often in a position to see this cycle happening to others, it is small wonder that they look a little askance at any new method which has its initiation in an aura of great excitement.

To avoid the dangers mentioned above, pathologists should avoid all reference to a "test" for cancer, with or without an eponymic. To say that a test for cancer is positive or negative leaves a degree of certainty in the mind of our clinical colleagues all out of proportion to what we are justified in saying. We should merely say that we looked for cancer cells in various secretions and exudates and report only that we found such cells or did not find them. Furthermore, since there are no pathognomonic criteria for cancer cells and

there are *degrees of certainty* concerning the nature of the cells we see, we might well use such qualifying phrases as "cells which might be cancer cells", "probably cancer cells," "almost certainly cancer cells". Such wording will force the clinician to evaluate the report in relation to everything else which he knows about the patient.

Examination for possible cancer cells in fluids is an important addition and supplement to already existing methods. It deserves not only use, but repeated use, when biopsy fails or is, for any reason, not available. If we are to avoid misuse and prevent abandonment of this valuable method we must guard against over-valuation of the certainty of its results and especially we must not neglect the other methods now in use.

Dr. W. Freeman, Fall River, Mass.: I am somewhat appalled by the suggestions implied during the discussion, namely, that in cases where positive malignant smears are found but no confirmation is obtained by x-ray or biopsy examinations, the suspected organ should be surgically removed anyway. In other words, it is implied that positive Papanicolaou smears are, in themselves, sufficient evidence of malignancy to warrant the removal of the suspected organ. The following case histories will, I am sure, show not only the fallacy of such a contention, but that it may also lead to catastrophe.

The first patient was a 72 year old, white woman who went to her family doctor because "she saw blood". He referred her to one of the surgeons with a diagnosis of "possible carcinoma of the womb". This aged woman, living alone, apparently was not very observing and did not give a good history but only repeated that she "saw blood" almost every day. Papanicolaou stains of pre-operative vaginal smears revealed a few clumps of scattered malignant tumor cells. As is our custom, I was present during the pre-operative examination under anesthesia. The cervix was smooth and shiny. The curetting procedure sounded as if the surgeon was scraping hard fibrous tissue and very little tissue was obtained. The Papanicolaou stained smears made directly from this material revealed no malignant neoplastic cells and this was later corroborated by histologic sections. At the same operation, he took five specimens from different locations in the cervix for biopsy and these were likewise negative. If we were to follow the suggestions made here, the surgeon would have performed a complete hysterectomy because of the diagnosis of carcinoma. Fortunately, however, we followed a more conservative course.

Four weeks later the patient re-entered the hospital upon our advice and we repeated the vaginal smears and again found small numbers of groups of malignant tumor cells. It was while I was discussing the stained smears with the surgeon that a student nurse called and said, "your patient just voided in a bedpan and fresh blood is present." A catheterized urine specimen was then obtained and the stained smears from the sediment revealed a great number of malignant neoplastic cells identical with those found in the vaginal smears. Intravenous and retrograde pyelograms revealed a nonfunctioning right kidney with obstruction at the ureteropelvic junction. This later proved to be en epidermoid carcinoma of the renal pelvis.

The second patient, a middle-aged, white man, a brother of one of my residents, developed a nonproductive cough persisting for months. X-ray studies revealed scattered lines and markings in the right lung which the roentgenologist considered as most likely produced by an interstitial pneumonitis. Papanicolaou-stained smears from the sputum contained suspicious cells which I interpreted to indicate the presence of malignancy. Stained smears made from material obtained by bronchoscopic examination contained malignant neoplastic cells. If we were to follow the suggestions made today, the right lung should have been removed. But at a later date, the chest x-ray films were almost negative. Not knowing from which lung the malignant cells were obtained, it was decided that smears should be made from each bronchus, using a different bronchoscope for each side. Cells identical with those found in the sputum and in the material obtained on the first bronchoscopy were found in the smears made from each bronchus. Today, a year later, this patient continues to have the same type of cells as found previously but as yet no x-ray or biopsy findings corroborate the diagnosis. We are still following the patient at regular intervals.

One must, therefore, come to this conclusion, namely, malignant tumor cells found on Papanicolaou stained smears, do not necessarily localize or identify the tumor. I am convinced that until the tumor is localized and/or identified by biopsy and/or x-ray, the surgical removal of the suspected organ is unwarranted.

Dr. S. M. Rabson, Fort Wayne, Ind.: There is one point that I want to raise regarding nomenclature, since nomenclature influences thinking just as the reverse is obviously true. Numerous speakers have used the word "early". It isn't clear to me what is meant by early, and I think that is very important in the evaluation of the Papanicolaou method of investigation. Dr. Papanicolaou said, when his case of carcinoma of the kidney was taken to the laboratory, that the pathologist stated it was the earliest carcinoma of the kidney that he had seen. Another speaker mentioned "early diagnosis". I don't know what an "early" carcinoma is. Does one mean a small area of carcinoma, does one mean the patient is not showing signs of cachexia, or does one mean by "early" carcinoma that the clinician has detected it perhaps sooner than he would have identified it by some other method?

Dr. G. N. Papanicolaou, New York, N. Y.: I think that in our diagnostic work based on the examination of smears prepared from sputum or from bronchial aspirates and washings, we have missed more positive cases than Dr. Herbut. Considering the cases in which the only material available for examination was sputum, our false negatives were approximately 25 per cent. I believe that in cases in which we had bronchial washings or aspirates as well as sputum, our statistics make a somewhat better showing. I am not prepared to give you more exact figures at the moment, but we are now engaged in the tabulation of a total of 1000 cases, which will give a more accurate idea of our results. We have always been more concerned with false positives than with false negatives, because of the greater significance attached to any positive report. However, we have been much more fortunate in our positive reports, more particularly in those of Class V. In approximately 70 reports of that class our accuracy was close to 100 per cent. We have had some false positives in Class IV, mostly in cases of bronchiectasis. Several major operations have been performed at New York and Memorial Hospitals chiefly on the strength of our Class V reports. The surgeon faces a difficult problem indeed when he receives a Class V report in a case in which other positive evidence is lacking. His responsibility in deciding in favor of a major operation chiefly on the strength of a positive smear report is undoubtedly great, yet he may be led to take this step when there is no possibility of confirming the smear findings by biopsy or in any other way. As an example, I may cite a case we recently had at the New York Hospital. The patient was a woman whose only symptom was hematuria from the right kidney. There was no loss of weight, nor any other symptom suggestive of malignancy. Pyelogram and x-ray films were essentially negative. Smears prepared from a ureteral specimen obtained from the left kidney were found to be negative, whereas those prepared from a specimen from the right kidney contained many typical carcinoma cells. Smears prepared from two subsequent specimens obtained from the right ureter were also found to be positive and were reported as Class V. In this case the surgeon decided in favor of a nephrectomy. An inspection of the kidney after its removal showed no evidence of tumor, nor any other distinct pathological lesion which could be recognized macroscopically. Upon receiving this report by the surgeon I was terribly shocked and I must confess that I had one or two sleepless nights until we received a report of the pathological findings, which proved the presence of a small intra-epithelial carcinoma of the pelvis of the kidney, extending into some of the collecting tubules. This case illustrates the problem with which the surgeon is confronted when he receives a positive smear report and has no means of confirming it, or what is even more difficult, when he receives a positive smear report and a negative biopsy.

UNIVERSAL SEROLOGIC REACTION WITH LIPID ANTIGEN

I. In Normal Persons*

REUBEN L. KAHN, Sc.D.

From the Serological Laboratory, University Hospital, University of Michigan, Ann Arbor, Michigan

The serologic reaction to be considered in this series of articles is unique in several respects. It is a precipitation reaction which has been found in all human beings and animals tested thus far; yet, it is differentiable both in different human beings and in animals. Each normal person gives a reaction of an individual serologic pattern and indications are that this pattern remains unchanged in health and assumes new characteristics in disease. This reaction has been studied thus far in syphilis, leprosy, malaria and tuberculosis. The results in normal human beings, in animals, in these four diseases and in others, as well as in special animal experiments, will be presented in this series of six articles and in later publications. These six articles will be brief and preliminary in nature because it is aimed to determine first the general behavior of the reaction in health and disease before undertaking fundamental studies of the reaction and of its detailed applicability to any given disease. In this article, the report will be limited to universal serologic findings in normal human beings.

The reaction under consideration is referred to as "universal serologic reaction"[16, 18] because experimental indications are that it is a widespread biologic manifestation. The antigen employed in the reaction, which consists of an alcoholic extract of heart muscle, is the same as that used in tests for syphilis; the technical steps, however, are entirely different. The reaction is based on a precipitation technic which combines different concentrations of sodium chloride, serum dilutions and incubation periods at cold temperature as variables. These three variables form the basis of the different serologic patterns noted under conditions of health and disease.

HISTORICAL

The foundation of the universal serologic technic began to be laid in this laboratory in 1922.[6] We then reported on the employment in serologic precipitation studies in syphilis (1) of a salt solution range extending from 0 to 2 per cent NaCl and (2) of incubation at cold temperature,[5] two basic steps in the universal technic. Those studies, however, were limited to syphilis. Among the several factors affecting precipitation which we investigated at that time was the effect of dilution on the formation of precipitates in mixtures of syphilitic serum and tissue extract antigen and we observed that NaCl solutions ranging from 0 to 2 per cent, when added in sufficient quantity to these mixtures, will interfere with

* This study has been conducted with the aid of a grant from the Office of Naval Research, Medical Sciences Branch, Navy Department, Washington, D. C.

Received for publication, October 28, 1948.

precipitation. In 1925 we observed[7] that the sensitivity of standard Kahn reactions with syphilitic serums is considerably reduced if the diluent of physiologic salt solution added to the serum-antigen mixture is replaced by water. This observation suggested that an increase in the sensitivity might be attained by the employment of salt concentrations above the physiologic level. This surmise proved true, and in 1928 we reported[10] that increasing the concentration of the salt solution above the physiologic level increases the sensitivity of the precipitation reactions with syphilitic serums until about 3 per cent NaCl is reached, when non-syphilitic serums begin to give weak reactions, while decreasing the salt concentration below 0.7 per cent decreases the sensitivity of the reactions with syphilitic serums. Yet, the increase in the NaCl concentration in the standard Kahn test in practice was limited to 0.9 per cent[9] in order to prevent oversensitive reactions.

Dunlop and Sugden[2] reported precipitation reactions with nonsyphilitic serum and Sachs-Georgi antigen by employing NaCl concentrations below 0.2 per cent, while with concentrations extending from 0.5 to 1.0 per cent, they obtained reactions only with syphilitic serums. Mackie and Anderson[20] obtained precipitation with syphilitic serums and various tissue extract antigens when employing NaCl concentrations below 2.5 per cent but not with nonsyphilitic serums. These workers were thus unable to corroborate the nonspecific precipitation findings reported by Dunlop and Sugden with low salt concentration.

In 1942 it was reported[12] that, under proper technical conditions, syphilitic and nonsyphilitic (false positive) serums employed with Kahn antigen suspension are affected differently by different NaCl concentrations; that an increase in salt concentration from 0 to 2.5 per cent increases the intensity of syphilitic reactions and decreases the intensity of nonsyphilitic reactions. This observation formed the basis of a practical technic as an aid in the differentiation between syphilitic and nonsyphilitic reactions. In the same year, Green and Shaughnessy[4] reported that nonsyphilitic human serums, giving negative reactions with the standard Kahn test, showed strong precipitation with Kahn antigen in a salt range of 0 to 0.3 per cent and weak precipitation in a salt range of 6 to 14 per cent NaCl after the tests had stood overnight; the reactions in salt ranges above 0.3 per cent and below 6 per cent they found to be negative. Syphilitic serums in their hands gave maximal precipitation between 2 and 8 per cent NaCl and practically no precipitation between 20 and 25 per cent NaCl. These authors also stated that, by the use of different salt concentrations, they obtained characteristic aggregation and dispersion patterns of the Kahn precipitate with serums from different animal species. Their experimental data are yet to be published.

In 1943 it was reported[16] from this laboratory that Kahn reactions given by rabbit, pig and cow serums became progressively stronger the lower the salt concentration employed. The NaCl concentration range was from 0 to 10 per cent. This behavior of animal serums was in contradistinction to that of syphilitic serums which had been found to give stronger Kahn reactions with increasing salt concentration. In 1947 it was also reported[17] that, by employing reduced

NaCl concentration such as 0.3 per cent in the Kahn technic, the serums from patients with malaria exhibit a far greater tendency toward precipitation than normal serums.

With regard to studies of the effect of incubation at cold temperature on precipitation with lipid antigen, we reported[5] in 1922 that mixtures of syphilitic serum and antigen suspension show least precipitation when incubated at ice box temperature, more marked precipitation when incubated at room temperature and most marked precipitation when incubated at 37 C. The period of incubation employed was four hours. A similar effect was noted in connection with Sachs-Georgi reactions by Sachs.[28]

The favorable effect of a short incubation period at warm temperature on syphilitic reactions was utilized for some time in the routine Kahn procedure.[8] Muckenfuss and Ebel[24] also reported on the effect of brief incubation periods on the sensitivity of Kahn reactions. At a temperature of 37 C. the sensitivity was increased, but not at ice box temperature. Prolonged incubation at ice box temperature tended to give false positives. Nagle and Lazarov reported[25] on a comparative study of overnight incubation at three temperatures of completed Kahn tests. They found that at 37 C. the precipitates tended to disperse, at room temperature they were unaffected, while ice box temperature rendered the tests oversensitive.

In 1940, we showed[11] that syphilitic and nonsyphilitic serologic reactions require different temperatures for optimal results; that warm temperature (37 C.) favors specific precipitation, while cold temperature, such as 1 C., favors nonspecific precipitation both with human and animal serums. This observation formed the basis of a verification technic as an aid in the detection of false positives. We then showed[15] that, by the use of an excessively sensitive tissue extract antigen and by the performance of the tests at 1 C., close to 100 per cent precipitation reactions in normal human beings and in different animals (hog, chicken, horse, cow and rabbit) could be obtained. Quantitative differential temperature studies in different animals were reported by Marcus,[23] and Carter[1] suggested the use of quantitative determinations in the author's verification technic.

In 1946 we reported[13] that it was possible to elicit nearly 100 per cent positive serologic reactions in normal human beings with antigens that gave specific reactions in tests for syphilis, such as Kolmer, Kahn and cardiolipin antigens, by certain technical steps which included low salt concentration combined with prolonged incubation at cold temperature. It was then decided to combine different NaCl concentrations ranging from 0 to 2.1 per cent, different serial dilutions of serum and different incubation periods at cold temperature in one serologic technic with a view of determining the type of reactions which would be given by human beings and animals. These efforts led to the standardized universal serologic technic described below.

UNIVERSAL SEROLOGIC TECHNIC

The universal technic consists of nine quantitative procedures, referred to as "set-ups", of different NaCl concentrations. Blood specimens are obtained in

the same manner as for serologic tests for syphilis. The serum is separated from the clot by centrifugation and is heated for 30 minutes at 56 C. before use. Nine NaCl solutions of the following concentrations are prepared: 0, 0.15, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 per cent. Serial serum dilutions are then prepared with each of these nine salt solutions in the following ratios: 1:1, 1:2.5, 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640. In some instances, the ratios of serum to salt solution employed extended to 1:200.

Tests are set up as for nine quantitative Kahns, but in which each quantitative employs serum dilutions with a solution of a given NaCl concentration. The first quantitative is carried out with serial dilutions of serum with water; the second, with 0.15 per cent NaCl solution and so on; and the ninth quantitative is carried out with serial dilutions of serum with 2.1 per cent NaCl solution. Kahn antigen suspension is prepared with 0.9 per cent NaCl solution in accordance with the Kahn standard technic. The ratio of serum dilution to antigen employed is 6:1. The antigen suspension is pipetted to the bottom of the tubes in 0.025 ml. amounts, followed by 0.15 ml. amounts of the dilutions of each serially diluted serum. The mixtures of serum dilutions and antigen suspension are then agitated for three minutes in the Kahn shaker after which 0.5 ml. of diluent is added to each tube, the contents mixed and the results read.

The diluent in each quantitative set-up consists of salt solution of the same concentration as was used in the preparation of the serum dilutions. The method of reading is similar to that employed in the Kahn test. Three readings are made. The first is made after mixing the reagents following the addition of the diluent to each tube. The second reading is made after four hours' incubation in the ice box (about 5 C.) and a third, after 24 hours in the ice box. Four plus, three plus or two plus readings are recorded as positive reactions, while one plus and borderline readings are recorded as doubtful reactions. In charting the results graphically, only the positive reactions were recorded.

When reading the results after incubation, it is well to remove one rack at a time from the ice box. This step will help retain the cool state of the serum-antigen mixtures until the actual reading time, as some precipitates will redisperse when the mixtures are unduly exposed to the warmth of room temperature. As each rack is taken from the ice box and brought to the reading table, a film of moisture will cover the tubes. To remove this film each tube is dipped in cold 95 per cent alcohol immediately before reading the precipitation results.

It should be added that, in the quantitative set-up of the universal technic in which the serums are serially diluted with water, we may be dealing with a situation in which the separation of globulin in the high dilutions might be a factor in precipitation with lipids. The interesting finding in that particular set-up is the absence of precipitation noted in the controls.

The universal serologic technic embraces a serodiagnostic technic for syphilis. Thus, the quantitative set-up with 0.9 per cent NaCl solution in which the results are read without incubation, is similar to the quantitative Kahn test. Hence, universal reactions can be readily correlated with serodiagnostic reactions for syphilis. If this quantitative set-up with 0.9 per cent salt solution shows precipitation with a given serum, that serum may be considered as giving also a

positive reaction with a test for syphilis. No suggestion of positivity for syphilis is noted in the table and charts presented in this article.

The universal serologic technic for the present is intended for research purposes. Indications are that it has potential value in serologic studies in health and disease. It is recognized that in its present form, the universal technic is quite elaborate, involving nine quantitative set-ups. To reduce the technical steps, five quantitative set-ups are being employed in this laboratory for investigative purposes in certain situations, as, for example, in tuberculosis. The salt concentrations used in these five set-ups are: 0, 0.3, 0.9, 1.5, and 2.1 per cent.

Only actual trial can best determine the optimal number of quantitative set-ups to employ in the investigation of the universal serologic response in a given disease. It is well, when beginning the investigation of the serologic response in a disease, first to employ the nine quantitative set-ups in order to note the more complete serologic pattern. A reduction in the number of quantitative set-ups can then be brought about without reducing the value of the results obtained.

In certain situations in animals, it may seem desirable to use special quantitative set-ups. For example, in experimental serologic studies in mice and guinea pigs, quantitative set-ups of 0, 0.15, 1.8 and 2.1 per cent NaCl solution might best be employed, since, as will be seen in the following article, the central zone of the universal reaction has consistently given negative precipitation results in these animals. However, these animals have as yet not been studied under pathologic conditions.

In recording the results of the universal serologic technic, it is suggested that the serologic readings be illustrated by graphs. Tabular data, it seems to us, do not bring out the characteristics of the serologic patterns as clearly as graphs.

UNIVERSAL SEROLOGIC REACTIONS IN DIFFERENT HUMAN BEINGS

The universal serologic technic was developed in this laboratory in the spring of 1946, and since then it has been continuously in use in human beings in health and in certain diseases and in animals under normal and experimental conditions. More than 400 universal reactions have been obtained in normal human beings but for reasons of economy of space only six reactions will be illustrated in this preliminary report.

Table 1 presents a universal reaction given by a normal individual. The nine quantitative precipitation set-ups employed, are arbitrarily divided into three zones. The three precipitation set-ups of the lowest salt concentrations (0, 0.15 and 0.3 per cent NaCl) are grouped under Zone I. The three precipitation set-ups close to physiologic salt concentrations (0.6, 0.9 and 1.2 per cent NaCl) are grouped under Zone II, while the three set-ups of the highest salt concentrations (1.5, 1.8 and 2.1 per cent NaCl), under Zone III.

As is shown in the table, no precipitation was obtained in the quantitative set-ups when the readings were made without incubation, with the exception of the three highest serum dilutions of the quantitative set-up in which water was used as diluent. After four hours' incubation, precipitation was obtained mainly in the quantitative set-ups of 0 and 0.15 per cent salt concentrations on the one

hand, and of 1.8 and 2.1 per cent salt concentrations on the other. After 24 hours' incubation, precipitation reached the quantitative set-ups of 0.3 and 1.5

TABLE 1

UNIVERSAL SEROLOGIC REACTIVITY WITH LIPID ANTIGEN

Quantitative precipitation results with increasing concentrations of NaCl solutions ranging from 0 to 2.1 per cent, employing normal (Kahn-negative) human serum.

SERIAL DILUTIONS OF SERUM	CONCENTRATIONS OF NaCl (PER CENT) USED IN PREPARATION OF SERIAL DILUTIONS OF SERUM								
	Zone I			Zone II			Zone III		
	0	0.15	0.3	0.6	0.9	1.2	1.5	1.8	2.1
Readings made without incubation of serum-antigen suspension mixture									
1:1	—	—	—	—	—	—	—	—	—
1:2.5	—	—	—	—	—	—	—	—	—
1:5	—	—	—	—	—	—	—	—	—
1:10	—	—	—	—	—	—	—	—	—
1:20	—	—	—	—	—	—	—	—	—
1:40	±	—	—	—	—	—	—	—	—
1:80	±	—	—	—	—	—	—	—	—
1:160	4	—	—	—	—	—	—	—	—
1:320	4	—	—	—	—	—	—	—	—
1:640	4	±	—	—	—	—	—	—	—
Readings after 4 hours' incubation in ice box									
1:1	4	4	2	—	—	—	2	4	4
1:2.5	4	4	±	—	—	—	±	4	4
1:5	4	4	±	—	—	—	—	2	4
1:10	4	4	—	—	—	—	—	2	4
1:20	4	4	—	—	—	—	—	2	4
1:40	4	4	—	—	—	—	—	2	4
1:80	4	4	—	—	—	—	—	4	4
1:160	4	4	—	—	—	—	—	4	4
1:320	4	2	—	—	—	—	—	4	4
1:640	4	—	—	—	—	—	—	4	4
Readings after 24 hours' incubation in ice box									
1:1	4	4	4	—	—	—	2	4	4
1:2.5	4	4	4	—	—	—	2	4	4
1:5	4	4	4	—	—	—	2	4	4
1:10	4	4	—	—	—	—	±	4	4
1:20	4	4	—	—	—	—	—	4	4
1:40	4	4	—	—	—	—	—	4	4
1:80	4	4	—	—	—	—	—	4	4
1:160	4	4	—	—	—	—	—	4	4
1:320	4	4	—	—	—	—	—	4	4
1:640	±	±	—	—	—	—	—	4	4

per cent salt concentration. These precipitation reactions are graphically illustrated in Figure 1.

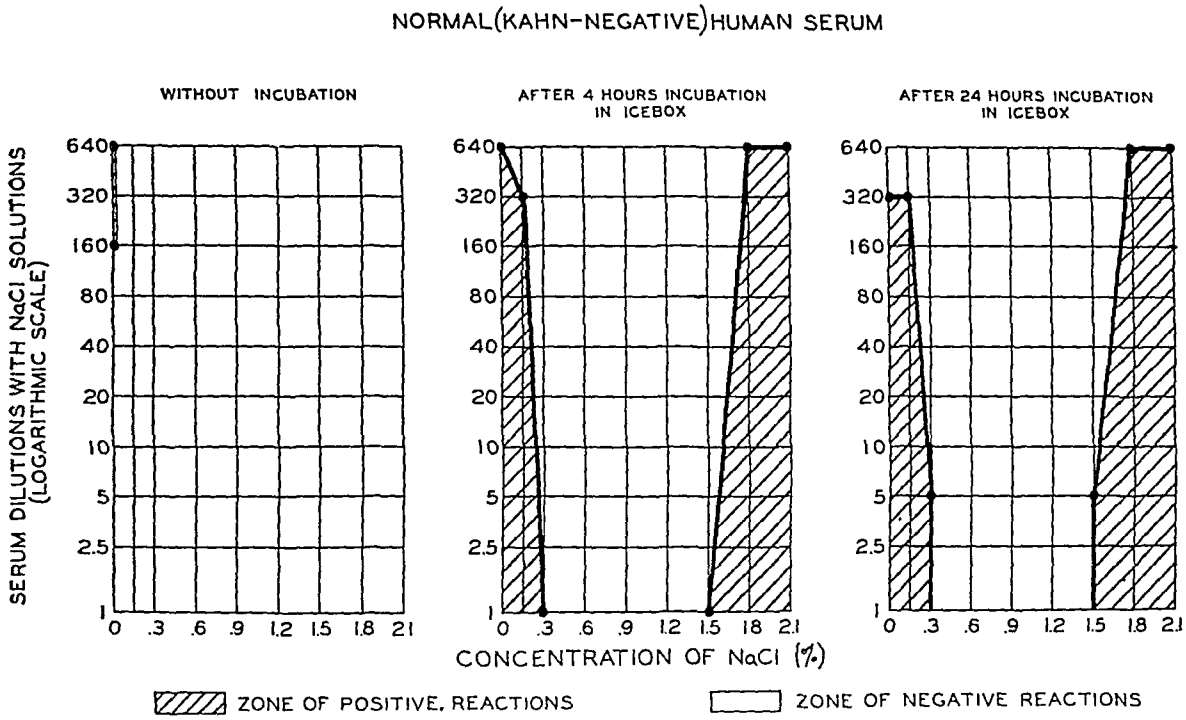
Figure 2 exhibits a serologic pattern similar to that of Figure 1 and is here presented to show that, while serologic patterns of different normal individuals generally differ from one another, now and then two serologic patterns are observed which are closely similar. A common characteristic of Figures 1 and 2 is that the degree of precipitation after four and 24 hours' incubation at cold temperature is approximately the same.

Figure 3 exhibits a serologic pattern different from the first two figures, especially in the fact that precipitation is most pronounced after 24 hours' incubation. No precipitation is noted without incubation. After four hours' incubation, precipitation is limited to Zone I. After 24 hours' incubation, precipitation is noted in all three zones. No increase in precipitation is noted in Zone I. In Zone II, precipitation reaches the serum dilution ratio of 1:2.5, while in Zone III, precipitation reaches the serum dilution ratio of 1:640.

A still different serologic pattern is illustrated in Figure 4. In Zone I, a similar degree of precipitation is noted both before and after incubation. In each of the three readings, precipitation reaches the serum dilution ratio of 1:640. No precipitation is noted in Zone II on immediate reading and after four hours' incubation. After 24 hours' incubation, precipitation reaches the serum dilution ratio of 1:2.5. In Zone III, considerable precipitation is noted immediately and on incubation, but only in relatively low dilutions. Precipitation reaches a dilution ratio of 1:10 without incubation, 1:20 after four hours' incubation and 1:40 after 24 hours.

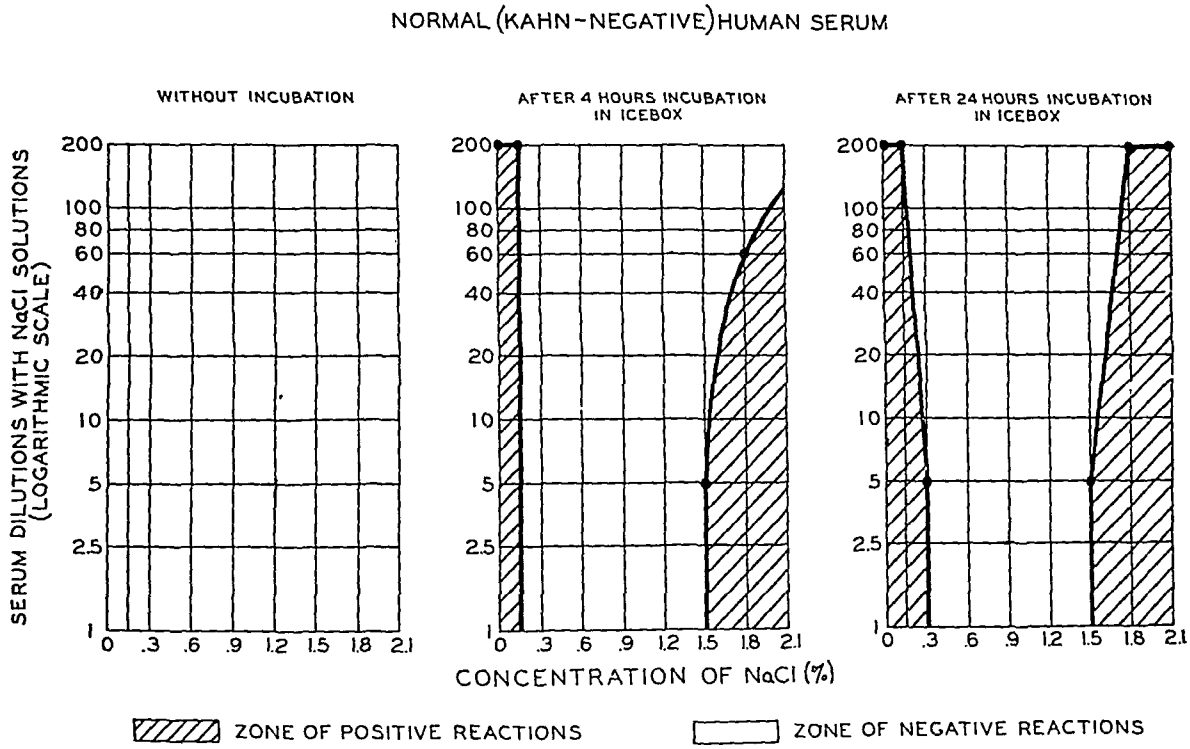
Figure 5 is characterized by practically no precipitation without incubation, by limited precipitation in Zones I and III after four hours' incubation and by considerable precipitation in all three zones after 24 hours' incubation. A still different serologic pattern is noted in Figure 6. Some precipitation without incubation is noted in Zone I. A relatively marked increase in precipitation is noted after four hours' incubation and only a slight increase after 24 hours' incubation. Of interest is the fact that precipitation after 24 hours' incubation illustrated in this chart is approximately the same as that illustrated in Figure 5. Yet, the precipitations on immediate reading and after four hours' incubation are altogether different in the two charts.

It must not be assumed that the universal reaction herewith considered is elicited only by standard Kahn antigen. Sensitized Kahn antigen, which is about 10 per cent more sensitive than standard antigen with syphilitic serums, gives broadly similar universal reactions. Somewhat more marked precipitation is obtained with this antigen than with standard antigen. An antigen prepared from powdered liver, instead of from powdered beef heart, was found to give reduced precipitation. Various lipid antigens are under investigation as well as various technics. The main reason the universal technic was standardized with Kahn antigen is that methods are available for maintaining the antigen at a standard level of sensitivity. This antigen has been employed in the universal technic for nearly three years with consistent results. Different lots of Kahn antigen have been found to give the same serologic patterns. Furthermore, each individual, under conditions of health, examined from time to time,



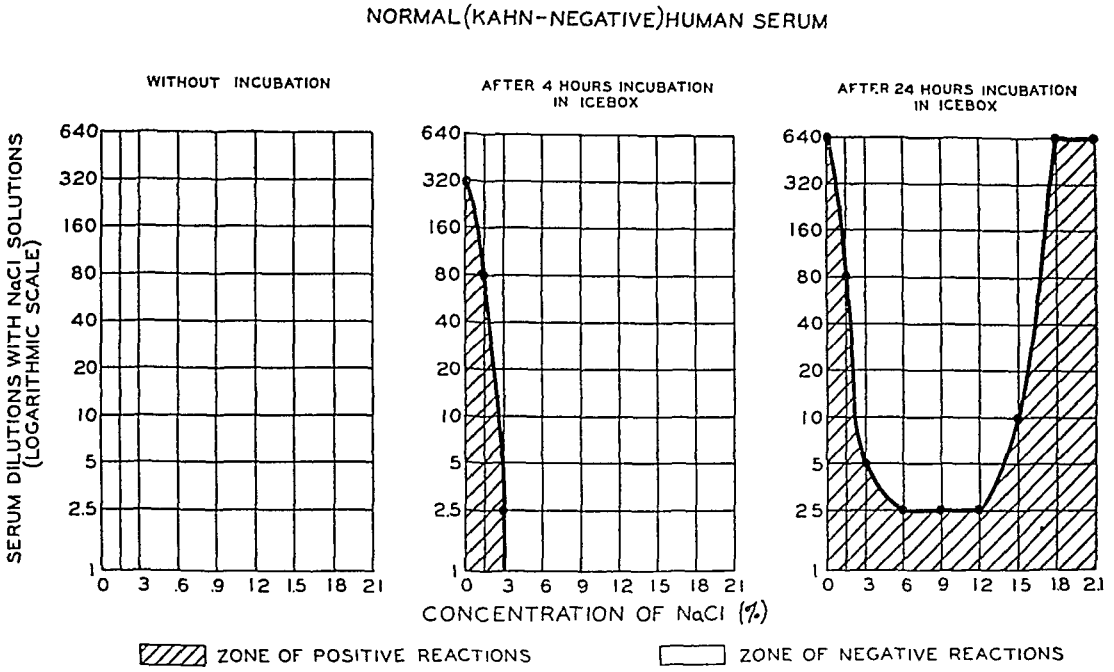
DK

FIG. 1. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.



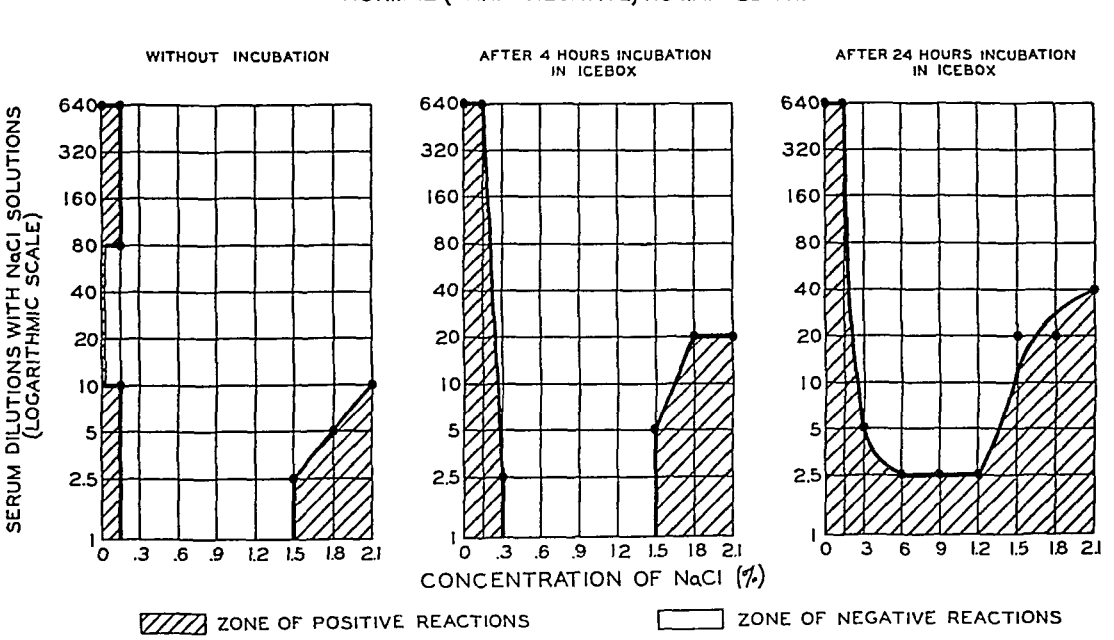
SP

FIG. 2. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.



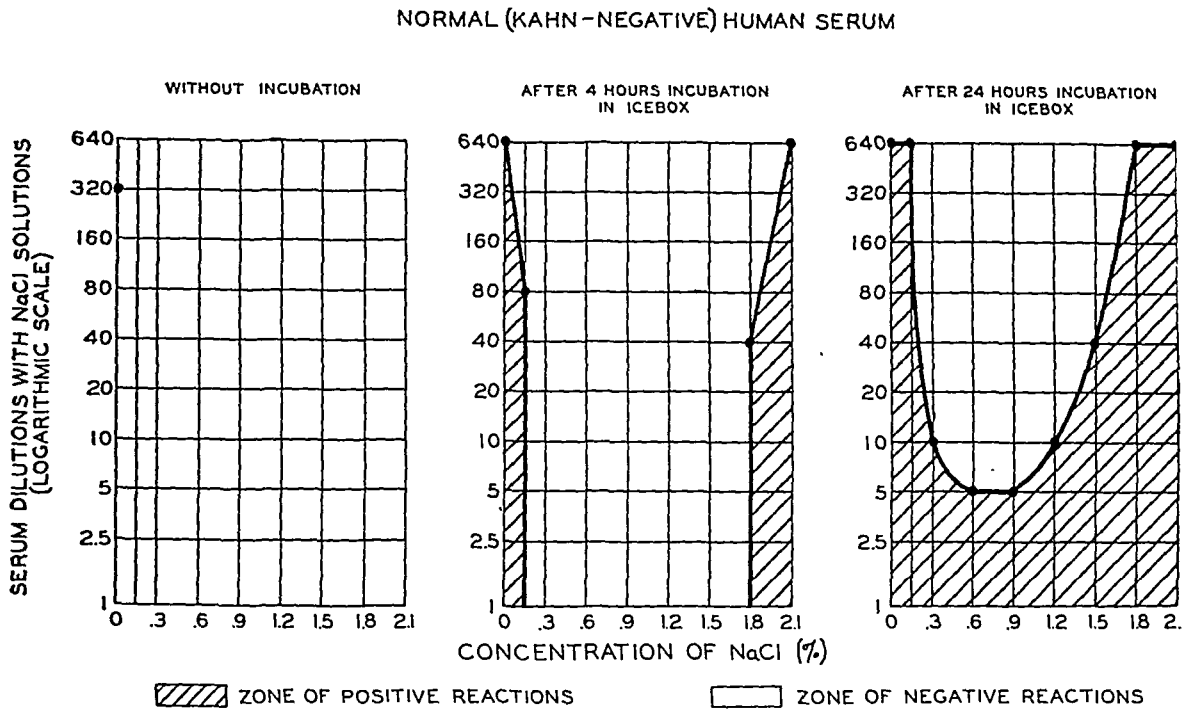
EL

FIG. 3. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.



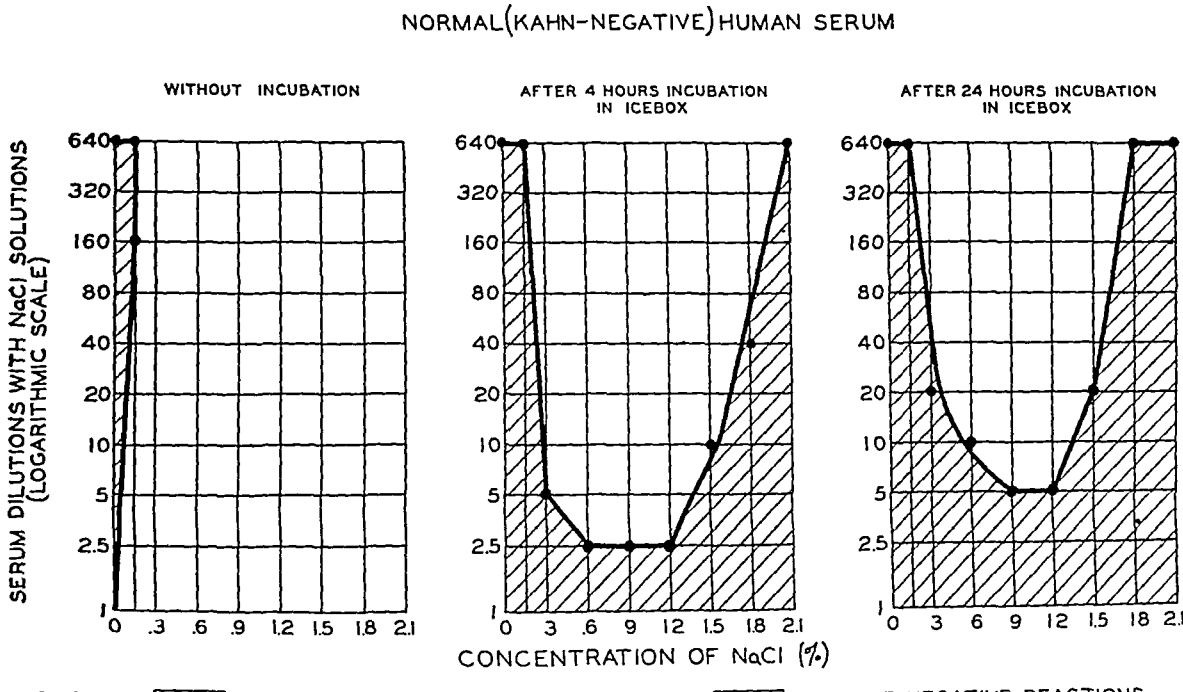
MS 1

FIG. 4. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.



GA I

FIG. 5. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.



AL I

FIG. 6. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

months apart, with different lots of Kahn antigen, has been found to give the same serologic pattern.

NATURE OF UNIVERSAL SEROLOGIC REACTION

A consideration of the nature of the universal serologic reaction with lipid antigen takes us into the realm of theory. At first, we believed that the reaction represented a colloid-chemical interaction between serum and lipids, perhaps unrelated to antigen-antibody reactions. Indeed, having devoted many years to the study of serologic reactions in syphilis with lipid antigen, it seemed difficult to believe that the very same antigen could give positive reactions with all serums tested. The possibility that these reactions are based on an unexplained artefact was also considered. Continued studies, however, led us to abandon these views in favor of a concept of antigen-antibody reactions.

As indicated above, each human being consistently gives an individual serologic pattern under conditions of health. That this pattern undergoes changes in certain diseases will be seen in the present series of articles. It would thus appear that we are dealing with a biologic reaction of a basic nature. Briefly, as a working hypothesis of the nature of universal serologic reactions in normal human beings, it is believed that, in the course of normal tissue wear and tear, lipids are liberated which act as antigens in stimulating the formation of specific antibodies and these react with tissue lipids *in vitro*.

Many explanations have been suggested as to the nature of serologic reactions in syphilis with lipid antigen. Eagle[3] believes that these reactions are not due to a general "state" of the serum proteins but to an actual substance which can be absorbed from the serum. Mackie and Watson,[21] in an extensive study on the nature of the Wassermann and Sachs-Georgi reactions, concluded that they are due to antibody-like substances homologous with antibodies natural to certain species of animals. The diagnostic reaction in syphilis they believed to be due to the nonspecific augmentation of this natural antibody-like substance. Ultramicroscopic precipitation studies carried out in this laboratory in 1931[22] led us to conclude that both syphilitic and nonsyphilitic human serums possess the property of precipitation when mixed with tissue extract antigen, syphilitic serums possessing this property to a marked degree and nonsyphilitic serums to a slight degree. More recently Neurath and associates[26] made comprehensive studies of protein fractions of serums giving specific serologic reactions in syphilis in relation to those giving false positive reactions. These workers believe that the antibodies in syphilitic human serum possess chemical and/or serologic properties which differ from the antibodies occurring in serum of false-positive reactors.

Sachs, Klopstock and Weil[27] injected in rabbits tissue lipids, the same as those used as antigen in tests for syphilis, and the animals gave negative complement-fixation and precipitation reactions for syphilis, but when the tissue lipids were first mixed with pig serum and the mixture injected in rabbits, the animals gave positive complement-fixation and precipitation reactions for syphilis. This

finding is in line with Landsteiner and Simm's observation[19] that alcoholic extracts of horse kidney (Forssman antigen) become antigenic when mixed with protein solutions, such as human or pig serum. Sachs, Klopstock and Weil suggested that tissue lipids, liberated as a result of tissue destructive processes in syphilis, in combination with spirochetal protein, serve as the antigen which stimulates antibody formation to lipids in syphilis.

However, theories which attempt to explain serologic reactions in syphilis ought to explain also the occurrence of these same reactions under normal conditions in animals and in a number of diseases other than syphilis in human beings. Actually, tests for syphilis give as high as 90 per cent of positive reactions with certain animal serums, such as horse and dog serums and perhaps 50 per cent with cow, chicken and rabbit serums. This, aside from the fact that serologic reactions are obtained also in various diseases in human beings in the absence of syphilis, and that in lepromatous leprosy these reactions are often stronger than those obtained in syphilis.

Recalling our lipid antigen-antibody hypothesis presented above, the universal serologic reaction given by normal human beings is the result of the presence of natural antibodies to tissue lipids in human serum. According to this hypothesis, the universal serologic reaction in different animals, considered in the following article of this series, is also the result of natural antibodies to tissue lipids. It follows that the positive reactions given by normal animals with serodiagnostic tests for syphilis are also the result of the same natural antibodies, since the serodiagnostic technic is embodied in the universal technic.

Insofar as the nature of serologic reactions in syphilis is concerned, the antibody theories which attempt to explain this reaction may be grouped in two categories. One theory assumes the existence of antibodies in syphilis which are distinctive from those of false-positive reactors, while the other assumes that the syphilis reaction represents an augmentation of naturally existing antibodies. The universal serologic studies suggest a theory which comprises both of these theories. The fact that normal human beings give universal serologic reactions would indicate the existence of a natural antibody to tissue lipids. In syphilis, as will be seen in article III of this series, (1) the universal reaction becomes intensified over the normal response, indicating a quantitative increase in antibody and (2) the same reaction also shows a distinctive serologic pattern, suggesting the presence of an antibody qualitatively different from the normal.

Let us now apply the same lipid antigen-antibody theory to universal serologic reactions in leprosy, malaria and tuberculosis, to be reported in the several articles of this series. In these diseases, acquired antibodies to lipids appear in the blood stream as a result of increased liberation of tissue lipids due to tissue destructive processes. These acquired antibodies cause the universal reaction to become quantitatively intensified over the reaction given by natural antibodies. This intensified reaction in some diseases may be so pronounced that precipitation "overflows" into the zone of the serodiagnostic reaction for syphilis.

In lepromatous leprosy, as in syphilis, the universal reaction is not only intensi-

fied quantitatively but it exhibits also some qualitative changes, as is evident from the distinctive serologic pattern obtained in this disease. This distinctive pattern suggests the liberation of particular lipids associated with this disease.

If the universal serologic reaction represents an antigen-antibody reaction, it would be expected that it conform to basic requirements of antigen-antibody reactions. Thus, any intensification of the normal universal reaction resulting from disease should be preceded by an incubation period. Actually, in malaria, for example, as will be seen later, a change in the normal universal reaction is not noted until about two to three weeks after the first attack of chills and fever.

Other factors affecting antigen-antibody reactions might be mentioned. Thus, it is well known that low resistance reduces the production of antibodies, also, excess of antigen interferes with such production. An illustration of both situations was observed in tuberculosis. While marked intensification of the universal reaction was noted in acute tuberculosis, no intensification was noted in prolonged chronic tuberculosis, and in terminal and miliary forms of this disease.

Has the universal reaction in normal human beings any clinical value? The answer is that the reaction was herewith presented primarily to demonstrate what appears to be a new serologic manifestation, and any clinical value it may have is yet to be established. It may be mentioned that, as indicated, the serologic patterns of the universal reaction exhibited in health undergo changes in the several diseases studied. Hence, in the investigation of these changes, the determination of normal patterns will obviously be essential as controls. Then again, if it be true that the universal reaction in health represents a reaction of natural antibodies to lipids, and the production of these antibodies is stimulated by lipids liberated in normal tissue wear and tear, then this reaction might prove to be of value as a serologic indicator of normal tissue wear and tear.

SUMMARY

A serologic reaction with lipid antigen is described which has been manifested by all normal human beings and animals tested thus far and which is referred to as "universal serologic reaction". The technic employed in eliciting this reaction is based on precipitation and combines serum dilutions, NaCl concentrations and incubation as variables. The reactions given by different normal persons are distinguishable within limits by individual serologic patterns.

REFERENCES

1. Carter, B. B.: The use of a quantitative test in verification procedures. Am. J. Syph., Gonor. and Ven. Dis., **26**: 629–631, 1942.
2. Dunlop, E. M., and Sugden, S.: The qualitative difference between syphilitic and non-syphilitic human serum in the syphilis flocculation test; a physico-chemical study of the Sachs-Georgi reaction. J. Path. and Bact., **39**: 149–163, 1934.
3. Eagle, H.: The Laboratory Diagnosis of Syphilis. St. Louis: The C. V. Mosby Co., p. 281, 1937.
4. Green, M. N., and Shaugnessy, H. J.: Effect of electrolytes upon Kahn precipitates from human and animal sera. Proc. Soc. Exper. Biol. and Med., **51**: 287–289, 1942.
5. Kahn, R. L.: A simple quantitative precipitation reaction for syphilis. Second Communication. Arch. Dermat. and Syph., **5**: 734–743, 1922.

6. KAHN, R. L.: Effect of dilution on the precipitation reaction for syphilis proposed by the author. Proc. Soc. Exper. Biol. and Med., **19**: 294, 1922.
7. KAHN, R. L.: Serum Diagnosis of Syphilis by Precipitation. Baltimore: The Williams & Wilkins Company, pp. 37 and 100, 1925.
8. *Ibid:* p. 141.
9. KAHN, R. L.: The Kahn Test, A Practical Guide. Baltimore: The Williams & Wilkins Company, p. 81, 1928.
10. KAHN, R. L.: Serum diagnosis of syphilis. In: Colloid Chemistry, Application to Biology and Medicine, edited by Jerome Alexander. New York: Chemical Catalogue Co., p. 761, 1928.
11. KAHN, R. L.: A serologic verification test in the diagnosis of latent syphilis. Arch. Dermat. and Syph., **41**: 817–830, 1940.
12. KAHN, R. L.: A new verification method in serology of syphilis. Univ. Hosp. Bull., Ann Arbor, **8**: 45–46, 1942.
13. KAHN, R. L.: Universal serologic reactivity with lipid antigens. Am. J. Pub. Health, **37**: 283–292, 1947.
14. KAHN, R. L.: Universal serologic reactivity with lipid antigen and its relation to serum reactions in syphilis. Abstracts of Communications, Fourth International Congress for Microbiology, Copenhagen, July 20–26, pp. 101–102, 1947.
15. KAHN, R. L., MARCUS, S., MCDERMOTT, E. B., AND ADLER, J.: A serologic (nonsyphilitic) reaction approaching universal sensitivity. J. Invest. Dermat., **5**: 459–462, 1942.
16. KAHN, R. L., MCDERMOTT, E. B., AND ADLER, J.: Effect of different salt concentrations on the Kahn reaction with animal sera. J. Bact., **45**: 73, 1943.
17. KAHN, R. L., WHEELER, A. H., AND ADAMS, C.: Studies on serology of malaria; malarial precipitation reactions with lipid antigen. J. Nat. Malaria Soc., **6**: 74–82, 1947.
18. KAHN, R. L., VILLALON, F. T., AND BARIBEAU, B. J.: Universal serological reactions with lipid antigen in leprosy. Proc. Soc. Am. Bact., pp. 84–85, 1947.
19. LANDSTEINER, K., AND SIMMS, S.: Production of heterogenic antibodies. J. Exper. Med., **38**: 127–138, 1923.
20. MACKIE, T. J., AND ANDERSON, C. G.: The precipitation reactions of normal serum and lipoid suspensions. J. Path. and Bact., **44**: 603–631, 1937.
21. MACKIE, T. J., AND WATSON, H. F.: On the immunologic nature of the principle in serum responsible for the Wassermann reaction, with reference also to the flocculation reaction of Sachs and Georgi. J. Hyg., **25**: 176–208, 1926.
22. MALLOY, A. M., AND KAHN, R. L.: The ultramicroscopic precipitation reaction in syphilis. J. Infect. Dis., **48**: 243–254, 1931.
23. MARCUS, S.: Some aspects of the Kahn reaction with animal sera and nonsyphilitic human sera. A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan, 1942.
24. MUCKENFUSS, R. S., AND EBEL, E.: The influence of temperature on the Kahn test. Am. J. Syph., **15**: 391–394, 1931.
25. NAGLE, N., AND LAZAROV, J.: Incubation of the completed Kahn test. Am. J. Pub. Health, **20**: 1216–1218, 1930.
26. NEURATH, H., AND ASSOCIATES: Biologic false positive reactions in serologic tests for syphilis, I–IV. Am. J. Syph., Gonor., and Ven. Dis., **31**: 347–468, 1947.
27. SACHS, H., KLOPSTOCK, A., AND WEIL, A. J.: Die Enstehung der syphilitischen Blutveränderung. Deutsche med. Wchnschr., **51**: 589–592, 1925.
28. SACHS, HANS (formerly of Heidelberg): Personal communication to the author.

UNIVERSAL SEROLOGIC REACTION WITH LIPID ANTIGEN

II. In Animals*

REUBEN L. KAHN, Sc.D., and BETTY J. BARIBEAU, B.S.

From the Serological Laboratory, University Hospital, University of Michigan, Ann Arbor, Michigan

It was stated in the previous article[1] that all human beings and animals tested gave positive universal reactions with tissue lipids as antigen. In this article, illustrative serologic patterns of these universal reactions given by animals of different species will be presented.

It had long been known that normal animals, such as cows, horses, pigs and rabbits give positive reactions with serologic tests for syphilis in which tissue lipids are employed as antigen. These tests thus have the paradoxical capacity of giving specific reactions for syphilis with human serums and nonspecific reactions with normal animal serums. However, it was shown in this laboratory that these reactions in syphilis and in animals have different characteristics. Thus, the reaction in syphilis is intensified by warm temperature, while the reaction in animals is intensified by cold temperature.[2] Also, the precipitates in the reaction in syphilis generally do not disperse in concentrated sodium chloride solution, while the precipitates in animal reactions generally disperse in such salt solution.[3] The data to be presented in this article and in the following one indicate that differences also exist in universal serologic reactions given by normal animals and by human beings with syphilis.

First, it is well to recall the relationship between the universal serologic reaction and the serodiagnostic reaction in syphilis. This relationship becomes evident on the examination of the universal serologic technic. Briefly, this technic consists of nine quantitative serologic tests, referred to as set-ups, with standard Kahn antigen. In each set-up, the serum is serially diluted with salt solution of a different NaCl concentration. These concentrations embrace 0, 0.15, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 per cent. Each serum dilution is mixed with Kahn antigen suspension in a ratio of 6:1. The results are then read after three minutes' agitation in the Kahn shaker and also after 4 and 24 hours' incubation in the ice box.

Those who are familiar with serodiagnostic tests for syphilis, will recognize one of the nine quantitative set-ups to be similar to the quantitative Kahn test for syphilis; namely, the one of 0.9 per cent NaCl concentration in which the results are read without incubation. The reaction obtained with this quantitative set-up may be said to be but one facet of the 27 facets of the universal serologic technic. It is this facet that has largely been investigated in animals thus far Let us now examine the entire universal picture in animals.

* This study has been conducted with the aid of a grant from the Office of Naval Research, Medical Sciences Branch, Navy Department, Washington, D. C.

Received for publication, October 28, 1948.

UNIVERSAL SEROLOGIC REACTION IN DIFFERENT ANIMALS

Serums of cows, horses, pigs, rabbits, dogs, guinea pigs and mice were examined with the universal serologic technic. The results obtained with a serum of each of these animals are best illustrated graphically by means of charts, the reason being that we are not dealing with a serologic reaction which is either positive or negative. We are dealing with a reaction which is always positive and the differences are manifested by different serologic patterns which are readily visualized by a graphic presentation of the results.

Cow serum. Eight cow serums were examined and the results obtained with one serum are presented in Table 1. The nine quantitative set-ups are arbitrarily divided into three zones. The set-ups with 0, 0.15 and 0.3 per cent NaCl concentrations are grouped under Zone I; the set-ups with 0.6, 0.9 and 1.2 per cent NaCl concentrations, under Zone II and the set-ups with 1.5, 1.8 and 2.1 per cent concentrations, under Zone III.

It is evident from the table that, in the first section in which the readings are made without incubation, the cow serum shows optimal precipitation with lipid antigen in Zone I in which the salt concentration is below that of the physiologic level; that the reaction extends to a lesser degree into Zone II in which the salt concentration is closer to the physiologic level and into Zone III in which the salt concentration is above that level. This serum gives a positive reaction with a sero-diagnostic test for syphilis in a dilution of 1:1, and in the same dilution, it is also positive up to the 2.1 per cent salt concentration. After 4 and 24 hours' incubation in the ice box, precipitation is increased in all three zones. A marked increase is noted in Zone II and the greatest increase is noted in Zone III.

The findings are graphically illustrated in Figure 1. A characteristic of this figure and of the others is that least precipitation is noted in Zone II which includes 0.9 per cent NaCl concentration. The increase in precipitation on incubation is generally most marked in Zones I and III in which the concentration of salt solution is below and above the physiologic level, respectively. In the case of Zone II of Figure 1, the increase in precipitation on incubation is manifested in definite increments. In terms of serum dilutions, the increase may be expressed by 1:1 without incubation, 1:2.5 after 4 hours' incubation and 1:5 after 24 hours' incubation.

Pig serum. Twelve pig serums were examined, and Figure 2 illustrates the serologic pattern of one serum. Marked precipitation is noted before and after incubation. The pattern is similar to that which might have been given by a syphilitic serum, considered in the following article. However, the pattern of a syphilitic serum, in most instances, shows no increase in precipitation on cold incubation in the 0.9 per cent NaCl set-up, while the pattern in Figure 2 shows an increase in this set-up, although the increase is not marked. In terms of serum dilutions, the range of the increase is from 1:5 to 1:10.

Rabbit serum. An examination of Figure 3 indicates that the serologic pattern given by rabbit serum with the universal serologic technic is essentially similar

TABLE 1

UNIVERSAL SEROLOGIC REACTIVITY WITH LIPID ANTIGEN

Quantitative precipitation results with increasing concentrations of NaCl solutions ranging from 0 to 2.1 per cent, employing cow serum.

SERIAL DILUTIONS OF SERUM	CONCENTRATIONS OF NaCl (PER CENT) USED IN PREPARATION OF SERIAL DILUTIONS OF SERUM								
	Zone I			Zone II			Zone III		
	0	0.15	0.3	0.6	0.9	1.2	1.5	1.8	2.1
Readings made without incubation of serum-antigen suspension mixture									
1:1	4	4	4	4	4	4	4	4	4
1:2.5	4	4	4	2	—	—	—	—	±
1:5	4	4	4	±	—	—	—	—	—
1:10	4	3	2	—	—	—	—	—	—
1:20	3	2	—	—	—	—	—	—	—
1:40	2	±	—	—	—	—	—	—	—
1:60	2	—	—	—	—	—	—	—	—
1:80	2	—	—	—	—	—	—	—	—
1:100	2	—	—	—	—	—	—	—	—
1:200	2	—	—	—	—	—	—	—	—
Readings after 4 hours' incubation in ice box									
1:1	4	4	4	4	4	4	4	4	4
1:2.5	4	4	4	4	4	4	4	4	4
1:5	4	4	4	2	—	—	—	4	4
1:10	4	4	4	—	—	—	—	2	4
1:20	4	4	3	—	—	—	—	1	3
1:40	4	3	2	—	—	—	—	±	2
1:60	4	2	±	—	—	—	—	—	—
1:80	4	2	—	—	—	—	—	—	—
1:100	4	—	—	—	—	—	—	—	—
1:200	4	—	—	—	—	—	—	—	—
Readings after 24 hours' incubation in ice box									
1:1	4	4	4	4	4	4	4	4	4
1:2.5	4	4	4	4	4	4	4	4	4
1:5	4	4	4	4	4	4	4	4	4
1:10	4	4	4	3	—	—	3	4	4
1:20	4	4	4	±	—	—	2	3	4
1:40	4	4	4	—	—	—	—	2	4
1:60	4	4	2	—	—	—	—	2	4
1:80	4	4	2	—	—	—	—	2	4
1:100	4	4	—	—	—	—	—	±	4
1:200	4	4	—	—	—	—	—	±	4

to that given by cow and pig serum. More than 50 rabbit serums were examined and all showed the characteristic increase in precipitation on incubation at cold temperature, common to nonsyphilitic serums.

Horse serum. As is evident from Figure 4, the serologic pattern of horse serum, based on eight examinations of different horse serums, is distinguishable

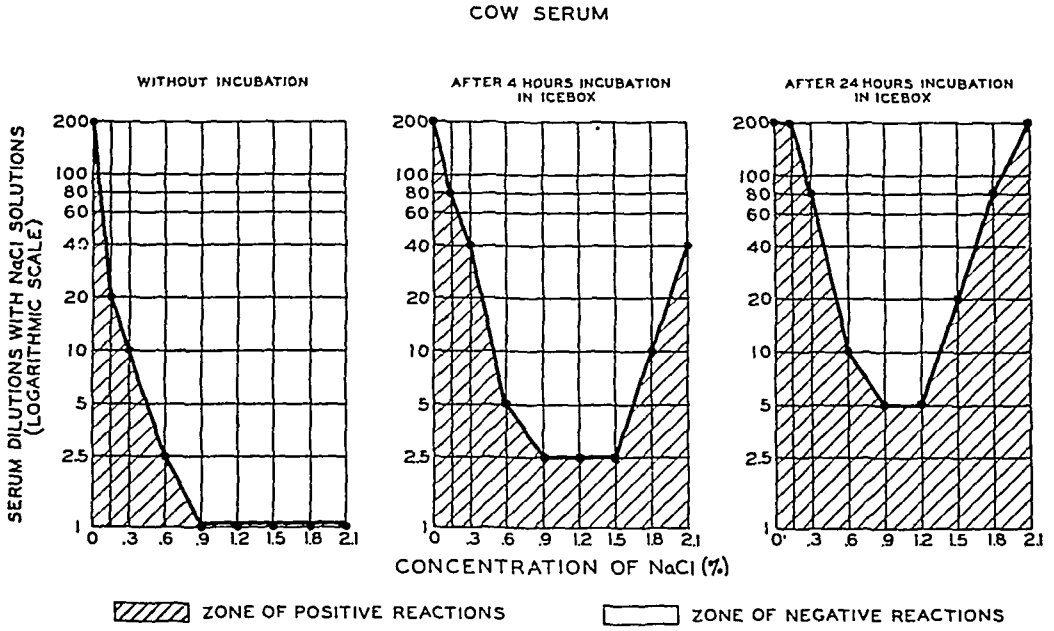


FIG. 1. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

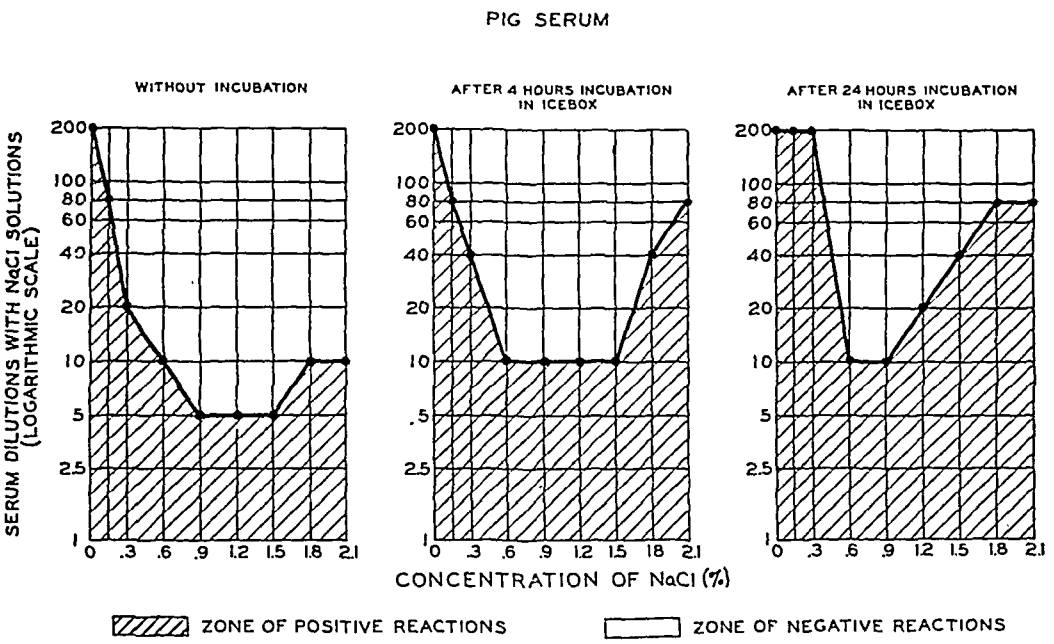


FIG. 2. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

from those given by the cow, pig and rabbit serums. Most marked precipitation is noted in Zone I with reduced salt concentration both before and after incuba-

tion at cold temperature. Zone II shows progressively increased precipitation on incubation. The same applies to Zone III but to a lesser degree.

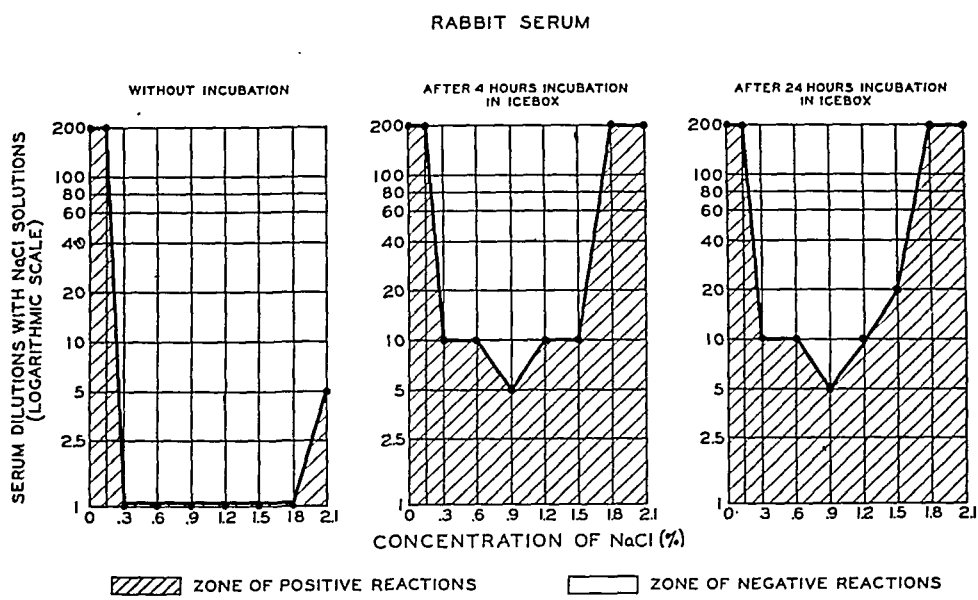


FIG. 3. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

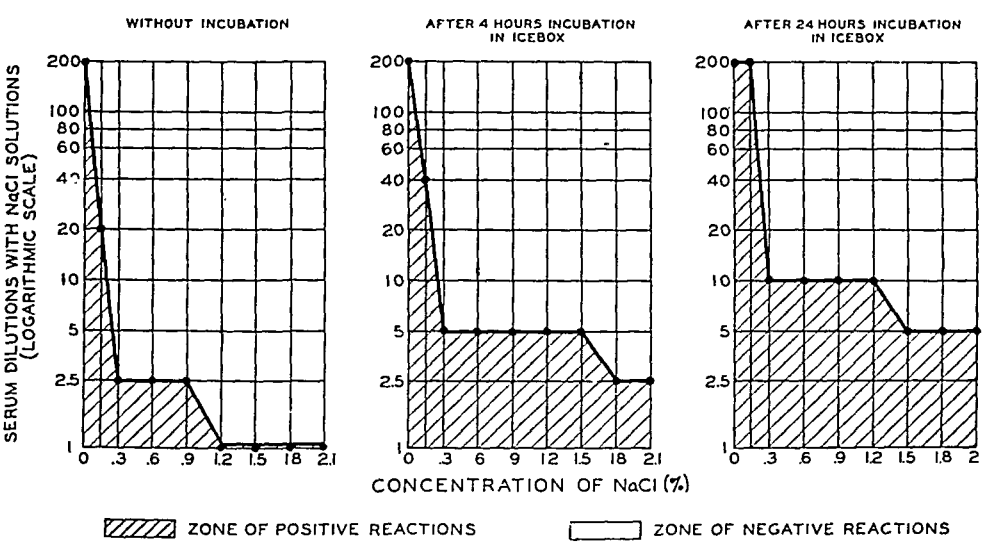


FIG. 4. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

Dog serum. Three dog serums were examined and it was found that they give reduced precipitation compared with cow, pig, rabbit and horse serums. The pattern illustrated in Figure 5 is similar to that commonly noted in man with the

exception of the precipitation in Zone I. Normal human serum generally does not show precipitation without incubation in the quantitative set-ups of 0 and

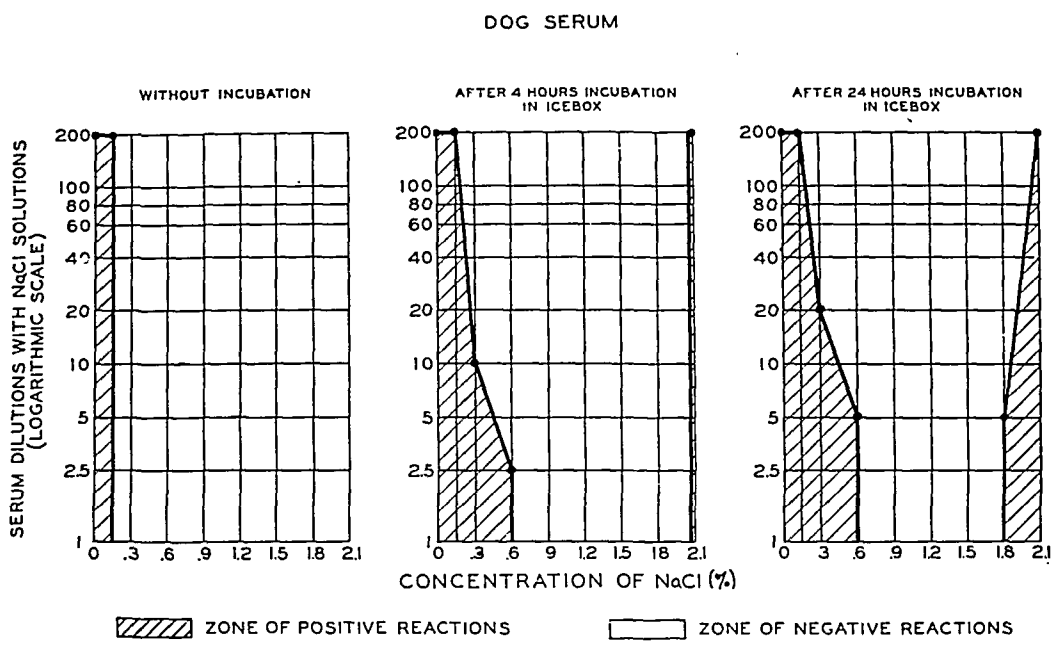


FIG. 5. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

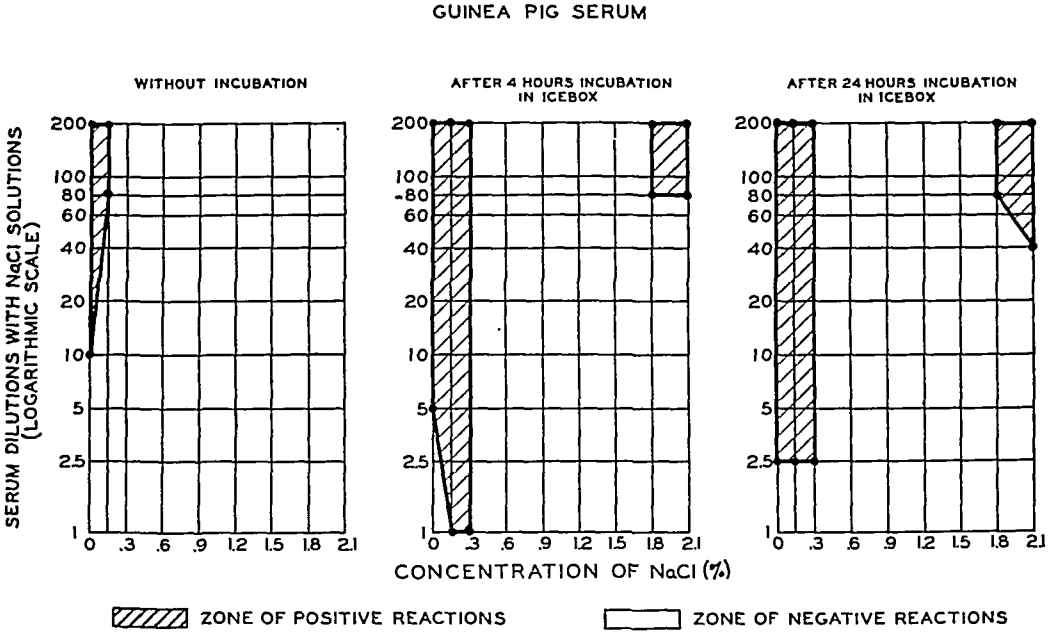


FIG. 6. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

0.15 per cent NaCl concentration in serologic patterns showing so little precipitation after 4 and 24 hours' incubation.

Guinea pig serum. Twelve guinea pig serums were examined and it was found

that they give a relatively weak and at the same time unique pattern compared with those given by the animals already described. A serologic pattern given by a serum is illustrated in Figure 6. Precipitation is noted in Zone I both before and after incubation. No precipitation is noted in Zone II and only in high serum dilutions in Zone III after incubation.

Mouse serum. Unique also is the serologic pattern given by mouse serum (Fig. 7). Relatively little precipitation is noted in Zone I. Only in the quantitative set-up with water is precipitation noted from 1:1 to 1:5 serum dilutions, not in the 1:10 to 1:40 dilutions, and then again in the 1:80 to 1:320 dilutions. No precipitation is noted in Zone II. In the case of Zone III, no precipitation is

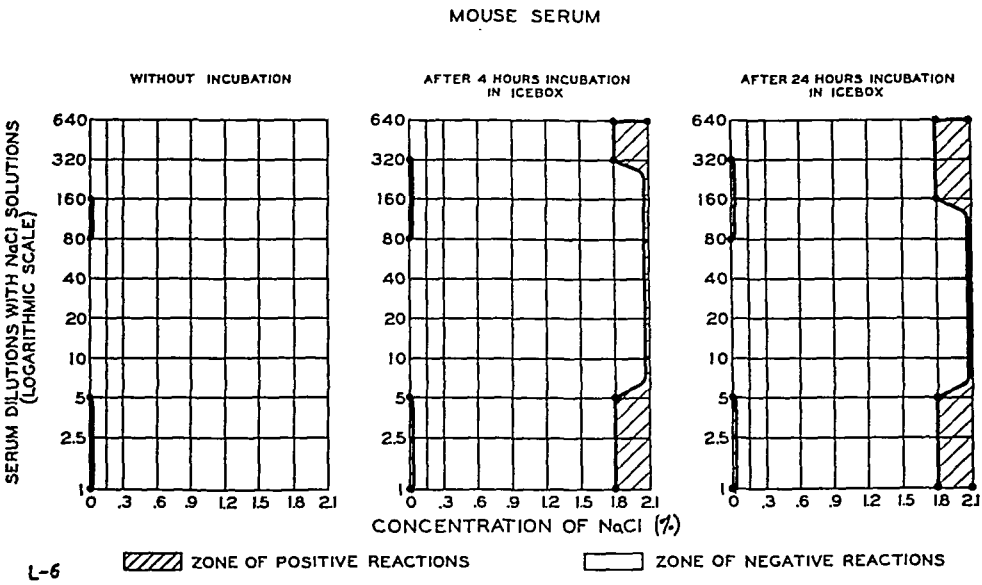


FIG. 7. Zones of precipitation, with NaCl concentrations ranging from 0 to 2.1 per cent, without incubation and after 4 and 24 hours' incubation in ice box.

noted without incubation. After 4 and 24 hours' incubation, precipitation is noted in the quantitative set-up of 2.1 per cent salt concentration, and only partially in the quantitative set-up of 1.8 per cent salt concentration, again only in the low and high serum dilutions. Pooled mouse serum had to be employed for these examinations, individual mice providing 0.5 to 1 ml. of blood. Six pooled blood specimens were examined. The question of the differentiation of different strains of mice by the universal serologic technic is under investigation.

The illustrative serologic patterns herewith presented give but a bird's-eye view of the behavior of different animal serums in the universal technic. Evidently, cow, pig and rabbit serums give similar serologic patterns, horse and dog serums give somewhat distinctive ones, while guinea pig and mouse serums give definitely distinctive patterns.

It is believed that the universal reaction in animals, as in human beings, represents a reaction between tissue lipid antigen and natural antibodies to tissue lipids. These lipids, liberated in the body in the course of tissue wear and tear,

serve as the stimulus for antibody production. The question of the production of antibodies in animals upon their injection with tissue lipids is under investigation; so also is the question of changes in the universal reaction in animals under conditions of disease.

SUMMARY

Illustrative serologic patterns of universal reactions with lipid antigen obtained with serums from different animals are presented. The serums studied were from cows, pigs, rabbits, horses, dogs, guinea pigs and mice. Cow, pig and rabbit serums were found to give similar serologic patterns. Horse, dog, guinea pig and mouse serums were each found to give distinctive serologic patterns.

REFERENCES

1. KAHN, R. L.: Universal serologic reaction with lipid antigen. I. In normal persons. Am. J. Clin. Path., **19:** 347–360, 1949.
2. KAHN, R. L.: A serologic verification test in the diagnosis of latent syphilis. Arch. Dermat. and Syph., **41:** 817–830, 1940.
3. KAHN, R. L.: Technique of standard Kahn test and of special Kahn procedures. Ann Arbor: University of Michigan Press, p. 31, 1946.

THE PATHOLOGIST AND CONTINUATION OF MEDICAL EDUCATION IN A PRIVATE HOSPITAL*

ARTHUR H. WELLS, M.D.

From the Department of Pathology, St. Luke's Hospital, Duluth, Minnesota

In many private hospitals throughout the country the pathologist has become the very backbone of medical education. His daily experiences with necropsies, clinical consultations, study of surgical specimens, clinical laboratory problems, and his close association with members of both the medical and resident staffs disclose to him a vast field of interesting scientific subjects not revealed to any of his medical colleagues. In addition to these experiences his neutrality among competitors, the exercise of honesty and capability reflected in his diagnoses, and the very nature of his work, all serve to place upon him the responsibility which he should accept of a leader in a program designed to keep the entire medical staff abreast of new developments in medicine.

The following comments, made as a result of certain personal experiences, include some suggested basic rules of procedure.

Practically any 200 bed general hospital has adequate subject material and qualified teachers for a postgraduate educational program of the first order. With careful planning, diplomacy and perseverance, the pathologist can render a real service through the proper organization and development of both the abundant medical subject matter and the frequently hidden teaching talents of the medical staff. To accomplish this end best, some fundamental rules of conduct are outlined for his adoption:

1. The pathologist should be an enthusiastic leader.
2. He should make classified notes of likely medical subjects as they present themselves.
3. Frequent consultation of the medical staff members and departments should lead to regular contributions to a well rounded program.
4. Interns and residents should present up to 50 per cent of the teaching program.
5. A fair and equal distribution of the work among the resident staff is mandatory.
6. Lectures, conferences, seminars, reviews of the literature, medical meetings, reviews of charts and demonstrations of patients must be scheduled at least one month in advance.
7. More complicated assignments, such as studies of series of cases and extensive reviews of the literature should be made several months in advance.
8. Routine weekly conferences must be scheduled for specified hours on regularly designated days.
9. A mimeographed bulletin of each monthly schedule including the titles, speakers, dates, times and places should have a complete distribution.

* Received for publication, November 26, 1949.

10. Participants should be notified three times: one month, one week, and one day in advance of their scheduled activity. Prodding at intervals may sometimes be necessary.

11. Insist upon a thorough preparation and a good delivery.

12. The material and methods of presentation must be varied and have practical value.

13. The liberal use of visual aids, including colored slides, gross specimens, projected outlines, blackboard drawings, presentation of patients, microscopic projections and moving pictures add greatly to the value and interest of presentations.

14. As far as possible, diagnostic and therapeutic problems should have been solved before presentation.

15. All serious diagnostic and therapeutic errors must be routinely discussed with complete frankness. Names of patients and physicians may be omitted.

16. The limitation of teaching assignments to a few men is detrimental and should not be tolerated.

17. The saturation point of the resident staff with the scheduled teaching program is first apparent when there is not sufficient time for the proper study and care of all patients admitted.

18. The pathologist should not dominate the program with pathologic presentations but should make an honest estimation of their relative importance.

19. Keep in mind that the larger the number of physicians making an effort to support the program, the more certain its success and the greater its value.

20. The long range educational benefit to the participating physicians is the principal criterion for selections of subjects and speakers.

21. Collaboration with adjacent institutions is likely to be mutually beneficial.

22. Constant criticism and variation of the program by the pathologist are necessary for continued improvement.

23. The democratic use of the counsel of the medical staff and of its committees for decisions on policy is important, but the pathologist should make his own decisions concerning details of procedure in those parts of the program for which he is responsible.

24. A sonic record and transcription of many of the presentations will be valuable for reference and publications.

25. Publications in medical journals of some of the material covered in the teaching program will add prestige and quality to the meetings.

Adherence to these basic rules should result in stimulation to greater effort and enthusiasm, a broader comprehension of the principles and problems of medicine, and a warmer appreciation of the interests and capabilities of one's colleagues. Although certain items are primarily designed for interns and residents all physicians should be invited to all presentations. We have found that our weekly Pathologic Conferences and Postgraduate Medical Conferences play a more important role in the medical education of our physicians than do the regular monthly staff meetings. Here a wide variety of subjects are covered, eventually including all practical aspects of the field of medicine. The principal sources of

this material include postmortem examinations, case studies and demonstrations of living patients, reviews of out-of-town medical meetings, reviews of outstanding articles from the medical literature, of recent advances in special fields and of selected topics in the basic sciences or in clinical medicine, clinical experimental studies and collective case reviews.

Other important factors in the postgraduate program in a private hospital include: (1) An informed and progressive hospital board of directors whose members constantly strive to keep pace with the rapid advances in medical science and appreciate the importance of continuous collaboration with the medical staff; (2) a community which has been educated over a long period by its physicians as to the value of postmortem examinations; (3) a system of flat rate fees for laboratory tests based upon the time spent in the hospital and not upon the amount of work performed, thus permitting all necessary laboratory studies to be made without throwing an undue financial burden upon the patient; (4) the help of a full-time medical anesthesiologist; (5) a medical library of adequate size; (6) a well-balanced rotating internship; (7) a guarded minimum amount of duty required of interns in the laboratory and blood bank, and in administration of routine parenteral fluids, the bulk of these tasks being performed by specially trained technicians and nurses; (8) a minimum of one intern for every 700 hospital admissions per year; and (9) of the greatest importance, a medical staff alerted to the mutual benefit to be derived from a continuous effort in giving personal teaching to interns and in collaborating with medical colleagues in all of the details of maintaining a high standard of medical practice in the community.

Experience teaches that pathologists are preëminently qualified for the role of leadership in the postgraduate medical training of their hospital medical staff.

THE CLINICAL EFFECT OF NITROGEN MUSTARD ON HODGKIN'S DISEASE*

LOWELL A. ERF, M.D., AND RICHARD D. BAUER, M.D.

From the Charlotte Drake Cardeza Foundation and the Department of Medicine, Jefferson Medical College and Hospital, Philadelphia, Pennsylvania

The nitrogen mustards came into prominence as possible therapeutic agents during the recent war.[15] Because of their demonstrable injurious activity to lymphocytes, they were subjected to further investigation and were found to exert a relatively specific toxic action upon certain neoplastic cells. The compounds have been shown to be of most value in the treatment of diseases and neoplasms of reticulo-endothelial origin, including lymphosarcoma, the leukemias, Hodgkin's disease, follicular lymphoblastoma and mycosis fungoides.

The toxicologic effect of the nitrogen mustards on the individual cell is quite similar to that of roentgen radiation.[12] We know that cells react in a variety of ways to nitrogen mustard, depending upon the type and stage of development of the cell and upon the amount and concentration of the chemical.[12] Alterations of these factors may produce inhibition of mitoses, abnormalities of chromosomes, mutations, excessive cellular enlargement, pyknosis, cellular "explosions" or lysis.[5] As yet we do not know the complete mechanism of action of the "mustard" within the cell but because of the observed changes we may assume that the nucleus is affected primarily and perhaps solely. Gilman and Philips[6] have shown that there is a release of hydrochloric acid intracellularly which probably accounts for some of the changes. Barron, Bartlett and Miller[1] have shown that nitrogen mustard inhibits certain enzymes, such as choline oxidase, choline esterase and pyruvate oxidase, which are necessary in cellular metabolism. It is possible that both of these factors contribute largely to the destruction of the cell. In addition there must be still other factors, as yet unknown, which account for the relatively specific effect of the chemical on certain neoplastic tissues. Even though death of a cell by the use of nitrogen mustard is strikingly similar to that brought about by means of x-ray irradiation, it is probable that the two agents alter cellular metabolism in entirely different ways.

Although there are hundreds of possible nitrogen mustards only a few have been studied to any great extent. The chemical action of the entire family is dependent upon the *bis*(beta-chloroethyl) group. The three compounds that are best known are the hydrochlorides of methyl *bis*(beta-chloroethyl) amine, ethyl *bis*(beta-chloroethyl) amine and tris(beta-chloroethyl) amine, designated as HN_2, HN_1 and HN_3 respectively. To date the most widely used has been HN_2.

Since 1942 numerous reports have appeared in reference to the use of HN_2—HCl in the treatment of diseases of the blood-forming organs.[4, 7, 11, 13, 14, 19, 20] Other investigators have adequately discussed the relationship between the

* Received for publication, December 3, 1948.

chemical constitution and the biologic action of the nitrogen mustards.[6, 15] From August 1946 and up to the present time, we have treated 150 patients with various types of lymphoma and allied conditions with nitrogen mustard.* It is our purpose to present here our findings in 43 of these patients who had Hodgkin's disease. The 43 patients received a total of 368 injections.

All diagnoses were made by biopsy. Consideration was given to the "blood picture" and previous x-ray therapy. The chemical was administered intravenously in physiologic saline solution in a dosage of 0.1 mg. per Kg. of body weight. Each milligram of the dry powder was dissolved in 1 ml. of the saline solution and was administered immediately since the compound deteriorates rapidly in solution. The total volume of a single dose varied from 5 to 10 ml. A series of

TABLE 1

NUMBER OF COURSES OF NITROGEN MUSTARD GIVEN TO PATIENTS

NUMBER OF COURSES ADMINISTERED TO PATIENTS (SINCE AUGUST 1946)	NUMBER OF PATIENTS	
	Living (November 1948)	Deceased
1	14	11
2	2	6
3	1	4
4	1	1
5	0	0
6	2	1
Total	20	23

four daily injections constituted a course of treatment. To date we have given as many as six courses of HN_2 to individual patients with Hodgkin's disease (Table 1).

RESULTS

A. Twenty-seven of the patients had received x-ray irradiation prior to the administration of nitrogen mustard therapy. Of these, 13 were considered to be x-ray-resistant and 14 were still deriving beneficial effects. Response to HN_2 varied considerably with the individual patient, but there was a definite parallelism in results obtained in the two groups (see Table 2). In both groups slightly less than one-third showed minimal improvement, which was characterized by partial relief of symptoms, for less than a week. Some of these patients died soon after the immediate effect of the drug wore off. About one-third showed a moderate to good clinical remission which lasted from four to six weeks. The remaining one-third of both groups showed good clinical remission for more than six weeks. These time limits are purely arbitrary and have been chosen merely

* The nitrogen mustard used in this series of cases was methyl *bis*(beta-chloroethyl) amine, and was obtained from Merck and Company, Inc., Rahway, N. J. through the courtesy of the National Research Council, Washington, D. C.

to facilitate classification. One of the patients in this group has been symptom-free for 14 months.

B. Sixteen patients in the series had never been subjected to previous irradiation. Three of these patients showed minimal improvement, 4 had remissions for a period varying from four to six weeks and 9 (over fifty per cent of the group) remained in remission for more than six weeks. One of these patients has had no relapse for 15 months.

TABLE 2

CLINICAL VALUE OF NITROGEN MUSTARD THERAPY ACCORDING TO PREVIOUS TREATMENT WITH X-RAY IRRADIATION

X-RAY IRRADIATION BEFORE NITROGEN MUSTARD TREATMENT	NUMBER OF PATIENTS	EFFECT OF NITROGEN MUSTARD TREATMENT			
		No Improvement	Minimal Improvement	Remission for less than 6 wk.	Remission for more than 6 wk.
X-ray given					
A. Effective	14	0	4	5	5
B. Resistant	13	2	3	5	3
X-ray not given	16	0	3	4	9

DISCUSSION

It may be significant that both of the patients who showed no improvement at all were in the x-ray-resistant group. This may possibly be explained by the fact that their disease was more advanced and of longer duration than the others. It may likewise be of importance that the greatest number of patients to show remissions for the longest period of time belonged in the group that had never before received x-ray irradiation. Karnofsky *et al.*[12] made observations which suggest that when x-ray is given first the cells become less sensitive to HN_2 and our experiences tend to substantiate this finding. In our own group of cases it does appear that there may be a greater possibility for a longer remission in a patient who has had no previous x-ray irradiation.

This series closely parallels that of Jacobson *et al.*[11] who reported his findings in 27 patients with Hodgkin's disease. In most of the patients who died the remissions had been shorter with each course of treatment. The remainder of the patients who died had had little or no remission following one course.

Hematologic Aspects

Much has been written concerning the "blood picture" after the administration of nitrogen mustard.[2, 4, 19] In general our series substantiates the findings in previous reports. The following effects were noted:

1. An initial drop in the lymphocyte count which returned to normal in from two to three weeks.
2. Similar findings in the monocyte series.
3. A fairly severe neutropenia reaching the lowest point in about two and one-half weeks and rising slowly thereafter.

4. A moderate drop in the platelet count about two weeks after therapy.
5. Occasionally a very slight decrease in the hemoglobin and red cell levels.

In 3 patients the treatment had to be stopped because of severe and sudden depression of one or more of the blood elements. One of these patients was a 33 year old white woman with generalized Hodgkin's disease who had had several courses of x-ray treatment prior to nitrogen mustard therapy. Gradually she had become x-ray resistant. Three courses of nitrogen mustard caused remission with no ill effects. During the fourth course her leukocyte count dropped from 10,000 to 200 cells per cu. mm. in four days. The red blood cell count showed very little change although anisocytosis and poikilocytosis were present. Her leukocyte count rose slowly during the following two weeks with the aid of several transfusions. Further treatment at a later date consisted of roentgen radiation. Four months later she died and autopsy revealed widespread Hodgkin's disease of lymph nodes and viscera. The second patient was a 68 year old white woman whose disease was complicated by diabetes mellitus and Kaposi's sarcoma. Her leukocyte count dropped from 4000 to 1900 after two injections of HN_2. The third patient was a white man whose white cell count suddenly dropped to 800. In all 3 patients, transfusions proved beneficial.

Recently investigators have been trying to regulate the dosage of nitrogen mustard in proportion to the leukocyte count, giving as much as 10 to 20 mg. injections if the white cell count is high.

In Craver's series[4] of 102 patients, many were treated with "double doses" and leukocyte counts as low as 1000 were frequently seen shortly after the fourth injection. The counts rose to their previous level during the following month.

Clinical Response

Of great interest is the effect of nitrogen mustard therapy on individual symptoms. By far the most prominent finding was the decrease in the size of the superficial nodes. It is probable that many of these nodes would not have shown typical Hodgkin's disease on biopsy but merely a lymphoid hyperplasia incident to the primary condition. In any case it was almost invariably the rule to see marked or moderate regression in the size of the nodes. Many of our patients had large masses, usually cervical or axillary, varying from 7 to 12 cm. in diameter, which actually "melted" away to 2 or 3 cm. or less within four days after the first dose of HN_2. Strangely, the biopsy sections before and after treatment were virtually identical. It is hard to explain this great decrease in tissue mass except by saying that there was massive cellular lysis, the cellular destruction including fibrocytes, eosinophils, lymphocytes, reticulum cells and giant multinucleated cells. Block *et al.*[2] have reported the same phenomenon in a series of patients with Hodgkin's disease in whom the nodes were subjected to biopsy at various intervals after the first injection of nitrogen mustard. Although the size of the nodes decreased appreciably there was little if any change in the microscopic appearance of the same node demonstrated by serial sections (Figs. 1 and 2).

The size of the palpable spleen was also reduced in most cases. Pain was

usually relieved and fever was reduced after treatment. Relief of cough was variable and closely paralleled reduction of the mass in the mediastinum responsible for the cough. Hepatomegaly was slowly and moderately reduced in fifty per cent of patients. Dysphagia occurred in 3 instances and was moderately relieved in 2. Dyspnea was greatly improved in two-thirds of subjects in whom it occurred. The drug proved beneficial in its quick relief of itching in the majority of cases. Little, if any, improvement was observed where the disease involved bone or where there was pleural effusion. Probably the most striking improvement was noted in 2 patients with central nervous system involvement. Both of these patients were white males, one an adult, the other a child. Both were delirious. The adult needed restraint because he would scratch and bite

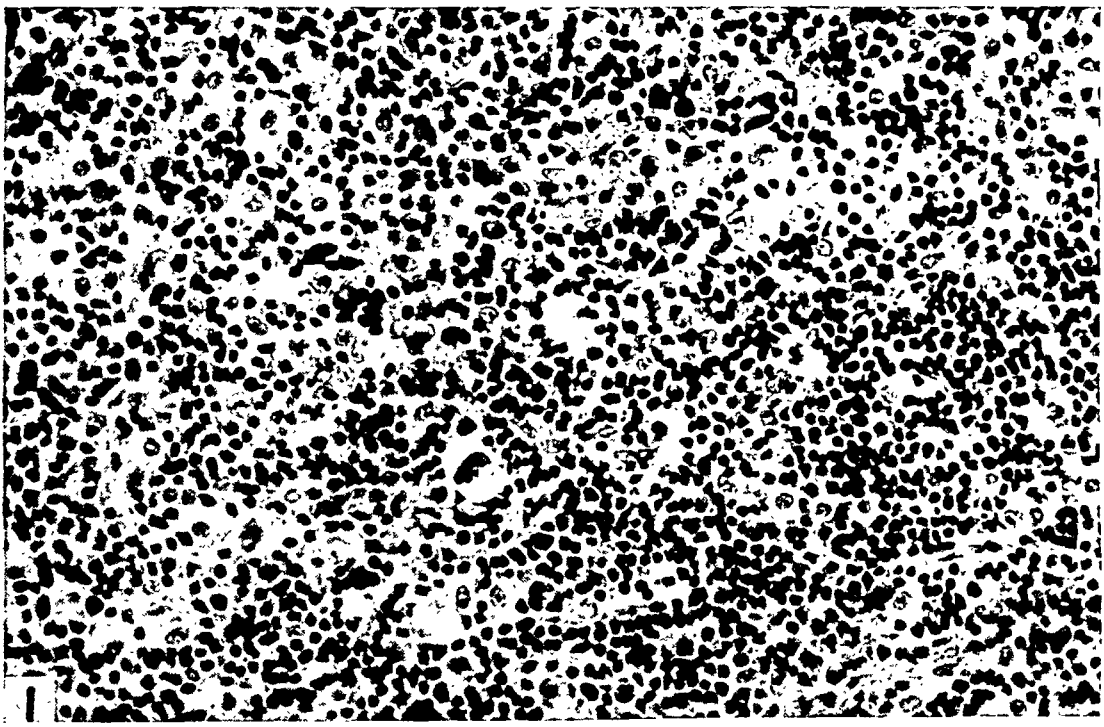
TABLE 3

EFFECT OF A SINGLE COURSE OF NITROGEN MUSTARD UPON VARIOUS SYMPTOMS AND SIGNS IN 43 PATIENTS WITH HODGKIN'S DISEASE

MANIFESTATIONS OF DISEASE	NO. OF PATIENTS	DEGREE OF IMPROVEMENT			
		None	Minimal	Moderate	Marked
Superficial nodes	30	1	2	15	12
Splenomegaly	16	2	1	9	4
Pain	14	2	2	3	7
Fever	11			5	6
Itching	7	1		2	4
Dyspnea	6	2		2	2
Hepatomegaly	6	1	2	3	
Cough	5	1	1	1	2
Pleural effusion	4		2	1	
Mediastinal mass	4		2	1	1
Dysphagia	3		1	2	
Bone	2	2			
Neurologic symptoms	2				2

any one who touched him. The child was unresponsive except to painful stimuli; it was necessary that 2 men hold him in order that a lumbar puncture might be performed. Within 12 hours after the first dose of nitrogen mustard, each patient was in full possession of his faculties and had no recollection of anything that transpired during his period of delirium.

Table 3 shows the effect of nitrogen mustard after a single course of treatment. After a second course, following relapse of symptoms, it was observed that in a few instances various manifestations of the disease did not respond as well or for quite as long a time. As subsequent courses of nitrogen mustard were needed, there was a definite diminution in the response of the individual symptoms from marked to moderate, from moderate to minimal, and from minimal to no improvement at all. This change in response was obvious in those patients who died after receiving two or more courses. The nausea and vomiting incident to



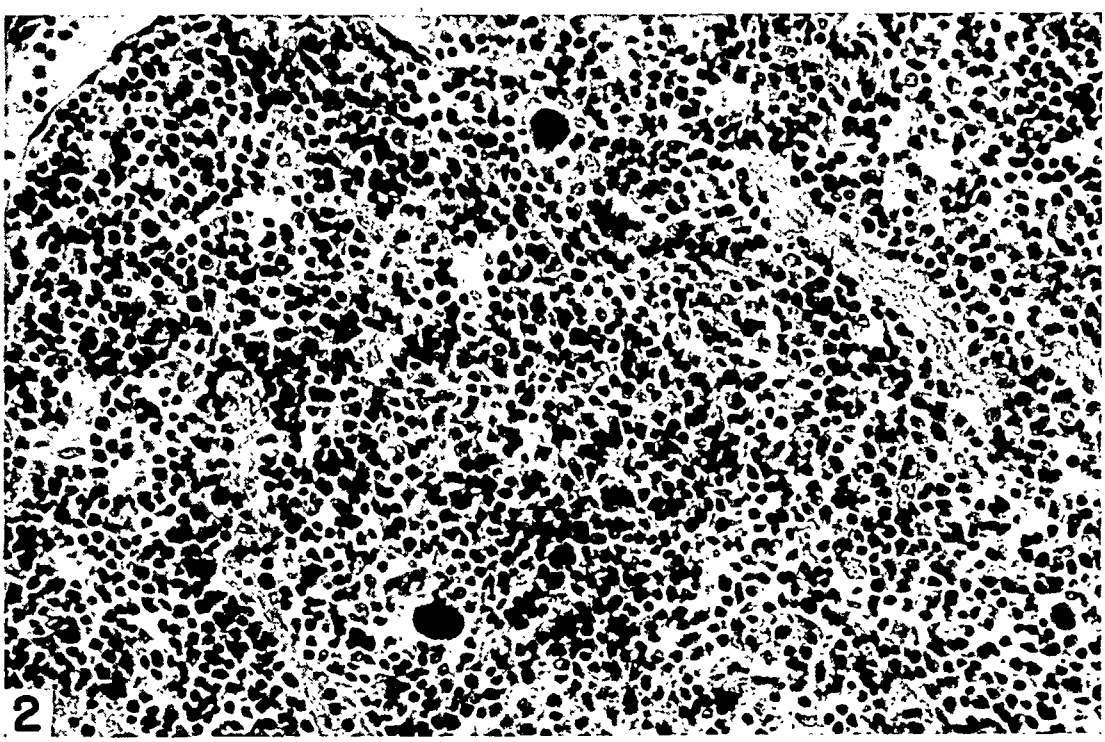


FIG. 1. Photomicrograph from section of lymph node from neck of a 17 year old Negro girl; mass measured 10 x 7 x 3 cm. Section was taken before treatment.

FIG. 2. Photomicrograph from section of same mass. Section was taken four days after the first injection of nitrogen mustard. The mass had then shrunken to 2 x 1 x 1 cm. Note similarity of general pattern in microscopic appearance to Figure 1.

treatment did not become more severe with repeated courses except in a few isolated instances.

The consensus is that nitrogen mustard in sufficient quantity and concentration is toxic to all cells but in weaker dilutions exerts its effect primarily upon cells of the lymphoid and reticulo-endothelial type. This effect was borne out by two of our patients in whom the Hodgkin's disease was complicated by Kaposi's sarcoma. After treatment with HN_2 the Hodgkin's lesion regressed while the Kaposi tumors became larger and showed no evidence of having been affected by the drug.

Although Hodgkin's disease responds to nitrogen mustard and x-ray irradiation in much the same way it cannot be said that nitrogen mustard is the treatment of choice in all instances. There are cases of Hodgkin's paragranuloma and a few of the granulomatous type that respond very well to surgery. Cases are on record of twenty-five and thirty-five year remissions in some instances in which small localized lesions had been completely excised.[10] It is seldom, however, that Hodgkin's granuloma would be considered for surgery as the treatment of choice and never has a case of Hodgkin's sarcoma been reported as responding to surgical removal. In general, roentgen radiation is most beneficial in localized lesions confined to limited areas, particularly the superficial ones. For generalized lesions our experience has shown that nitrogen mustard is the treatment of choice except where bone is involved. On the other hand Low-Beer[15] believes that, in his hands x-ray radiation of the whole body has proved as beneficial as HN_2 in generalized Hodgkin's disease. His method is to give weekly treatments of 5 to 10 r from a distance of eight feet. The question arises as to whether there is any indication for the combined use of the chemical with x-ray radiation at the same time. None of our patients have undergone combined therapy of this nature, but like Craver, we feel that the benefits obtained are not worth the risk of the combined side-effects of the two agents.

CONCLUSIONS

The use of nitrogen mustard in Hodgkin's disease seems to be particularly indicated:

1. When the lesions are generalized,
2. When the lesion, whether localized or generalized, is resistant to x-ray, and
3. In the presence of generalized symptoms, such as fever, itching and neurologic manifestations, which are particularly amenable to chemotherapy.

N. B. It is desirable to hospitalize patients who are to receive nitrogen mustard therapy, in order to take frequent blood counts, and give supportive treatment for such symptoms as severe protracted nausea or vomiting.

SUMMARY

1. In 43 patients with Hodgkin's disease a total of 368 injections of nitrogen mustard was given. Two-thirds of the group had had previous x-ray therapy.

2. Among the patients who had had no previous x-ray irradiation, there was

a greater number (60 per cent) who had remissions and these remissions lasted longer (6 weeks or more) than in patients previously irradiated.

3. The most marked improvement noted following nitrogen mustard was regression in size of nodes. In a lesser number of cases there was also decrease in size of spleen and remission of pain, fever and itching.

4. As subsequent courses were needed, there were observed fewer and shorter remissions in all symptoms.

5. Nitrogen mustard may produce serious depression of the blood-forming elements.

6. Nitrogen mustard is the treatment of choice in patients with generalized Hodgkin's disease and/or where the disease is resistant to x-ray.

7. Whole-body irradiation is said to be just as beneficial as HN_2 in generalized Hodgkin's disease.

REFERENCES

1. BARRON, E. S. G., BARTLETT, G. R., AND MILLER, Z. B.: Effect of nitrogen mustards on enzymes and tissue metabolism. J. Exper. Med., **87:** 489–519, 1948.
2. BLOCK, M., SPURR, C. L., JACOBSON, L. O., AND SMITH, T. R.: Histopathologic effects of nitrogen mustard therapy upon normal and neoplastic hematopoietic tissues. Am. J. Clin. Path., **18:** 671–689, 1948.
3. BURCHENAL, J. H.: The newer nitrogen mustards in the treatment of leukemia. Radiology, **50:** 494–499, 1948.
4. CRAVER, L. F.: The nitrogen mustards; clinical use. Radiology, **50:** 486–493, 1948.
5. FRIEDENWALD, J. S., BUSCHKE, W., SCHOLZ, R. O., AND MOSES, S. G.: Some effects of sulphur and nitrogen mustards on cell nuclei in mammalian cornea. Approaches to Tumor Chemotherapy, pp. 76–84, A. A. A. S., Washington, 1947.
6. GILMAN, A., AND PHILIPS, F. S.: Biological actions and therapeutic applications of the β-chloroethyl amines and sulfides. Science, **103:** 409–415, 1946.
7. GOODMAN, L. S., WINTROBE, M. M., MCLENNAN, M. T., DAMESHEK, W., GOODMAN, M. J., AND GILMAN, A.: Use of methyl bis(beta-chloroethyl) amine and tris(beta-chloroethyl) amine ("Nitrogen mustards") in the therapy of Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. Approaches to Tumor Chemotherapy, pp. 56–64, A. A. A. S., Washington, 1947.
8. GRAEF, I., KARNOFSKY, D. A., JAGER, V. B., KRICHESKY, B., AND SMITH, H. W.: The clinical and pathological effects of nitrogen and sulphur mustards in laboratory animals. Am. J. Path., **24:** 1–47, 1948.
9. HOSTER, H. A., DRATMAN, M. B., CRAVER, L. F., AND ROLNICK, H. S.: Hodgkin's disease, 1832–1947. Cancer Research, **8:** 1–78, 1948.
10. JACKSON, H., JR., AND PARKER, F., JR.: Hodgkin's disease and allied disorders. New York: Oxford University Press, p. 89, 1947.
11. JACOBSON, L. O., SPURR, C. L., BARRON, E. S. G., SMITH, T. R., LUSHBAUGH, C., AND DICK, G. F.: Nitrogen mustard therapy; studies on the effect of methyl-bis(beta-chloroethyl) amine hydrochloride on neoplastic diseases and allied disorders of the hemopoietic system. J. A. M. A., **132:** 263–271, 1946.
12. KARNOFSKY, D. A., BURCHENAL, J. H., ORMSBEE, R. A., CORNMAN, I., AND RHOADS, C. P.: Experimental observations on the use of nitrogen mustards in the treatment of neoplastic diseases. Approaches to Tumor Chemotherapy, pp. 11–23, A. A. A. S., Washington, 1947.
13. KARNOFSKY, D. A., CRAVER, L. F., RHOADS, C. P., AND ABELS, J. C.: An evaluation of methyl bis(beta-chloroethyl) amine hydrochloride and tris(beta-chloroethyl) amine hydrochloride (Nitrogen mustards) in the treatment of lymphomas, leukemia and allied diseases. *Approaches to Tumor Chemotherapy*, pp. 37–55, A. A. A. S., Washington, 1947.
14. KARNOFSKY, D. A., GRAEF, I., AND SMITH, H. W.: Studies on the mechanism of action of the nitrogen and sulphur mustards in vivo. Am. J. Path., **24:** 275–290, 1948.
15. LOW-BEER, B. V. A. (Radiotherapist at the University of California): Personal communication.
16. PHILIPS, F. S., AND GILMAN, A.: The relation between the chemical constitution and

the biological action of the nitrogen mustards. Approaches to Tumor Chemotherapy, pp. 3–10, A. A. A. S., Washington, 1947.
17. Rhoads, C. P.: Nitrogen mustards in the treatment of neoplastic disease. J. A. M. A., **131:** 656–658, 1946.
18. Smith, T. R., Jacobson, L. O., Spurr, C. L., Allen, J. G., and Block, M.: A coagulation defect produced by nitrogen mustard. Science, **107:** 474, 1948.
19. Spurr, C. L., Jacobson, L. O., Smith, T. R., and Barron, E. S. G.: The clinical application of methyl bis(beta-chloroethyl) amine hydrochloride to the treatment of lymphomas and allied dyscrasias. Approaches to Tumor Chemotherapy, pp. 24–36, A. A. A. S., Washington, 1947.
20. Wintrobe, M. M., McLennan, M. T., and Huguley, C. M., Jr.: Clinical experiences with nitrogen mustard therapy. Approaches to Tumor Chemotherapy, pp. 65–75, A. A. A. S., Washington, 1947.

EFFECT OF CARONAMIDE ON EXCRETION OF PHENOLSULFONPHTHALEIN*

WILLIAM P. BOGER, M.D., AND J. WILLIAM CROSSON, M.D.

From the Philadelphia General Hospital and Germantown Hospital, Philadelphia, Pennsylvania

Phenolsulfonphthalein (phenol red, PSP) is commonly employed to measure renal function, and at the low plasma concentrations resulting from the intravenous or intramuscular injection of 6 mg. (1 ml. of commercially available PSP solution), from 94 to 96 per cent of this dye is excreted into the urine by the renal tubules.[13] It has been shown that PSP, diodrast, para-aminohippurate (PAH), and penicillin[2, 11, 14] are excreted into the urine by the same renal tubular excretory pathway. Caronamide† has been shown to inhibit the excretion of penicillin in dogs[1] and in man,[4, 9] and of para-aminohippurate[8] and PSP[3] in dogs. Only casual reference has been made in clinical literature to interference with the excretion of PSP.[5, 15] Because the PSP test is used so widely, it seemed pertinent to determine (a) if caronamide does inhibit the excretion of PSP; in the event that it does, (b) if a reduction in PSP clearance produced by caronamide is an indication of the temporary and reversible inhibition of a tubular transport-mechanism or an evidence of renal impairment; and (c) if such a reduction in PSP clearance might serve as a simple means of determining indirectly the effectiveness of caronamide administered according to a given schedule, in inhibiting the excretion of penicillin. The study here reported was carried out in an effort to answer these questions.

PRELIMINARY EXPERIMENT

Method

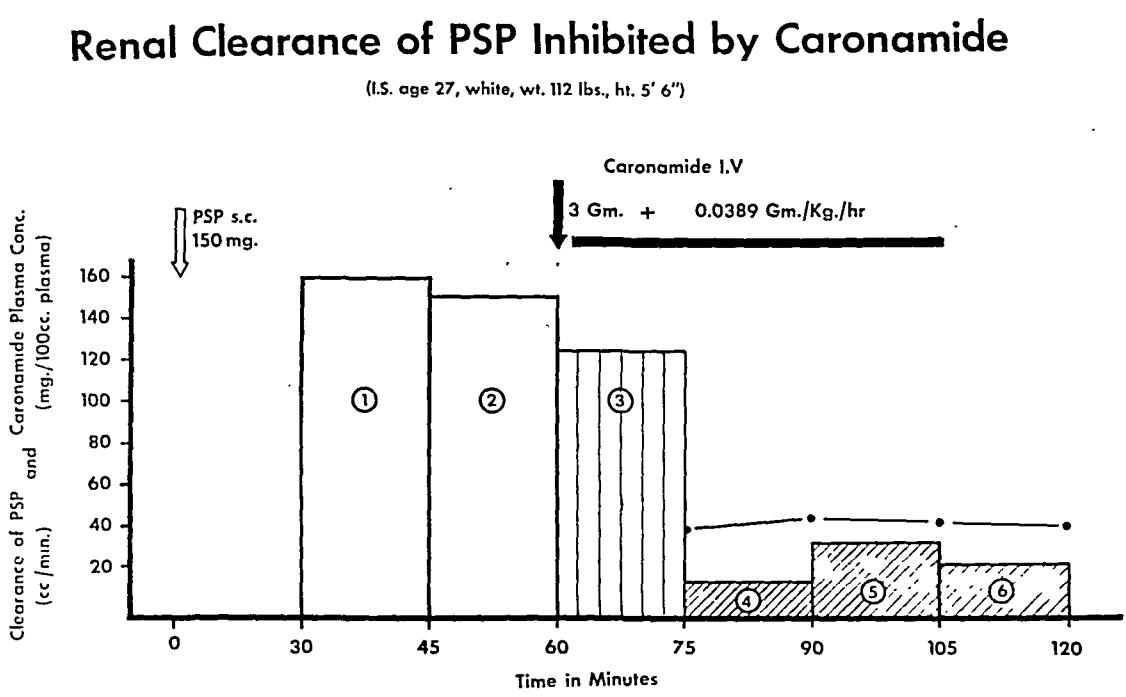
In order to determine whether or not caronamide inhibited PSP excretion, a renal clearance of the dye was performed in the following way. Patient IS, a 27 year old white woman, convalescent from infectious arthritis, was given a subcutaneous injection of 150 mg. of PSP (25 ml. of the commercially available solution of dye). At 30 minutes the bladder was emptied and washed with sterile saline solution through an inlying catheter. Two 15-minute urine collections were made and the patient was then given 3 Gm. of caronamide intravenously. Upon the completion of this injection an infusion of caronamide, at the rate of 0.0389 Gm. per kilogram per hour, was started and continued while four additional 15-minute collections of urine were made. At the beginning and end of each period of collection of urine, samples of blood were drawn for the determination of the concentration of PSP in the plasma, the last four blood specimens being used also for the estimation of the concentration of caronamide in the plasma.

* Received for publication, December 13, 1949.

† 4′-carboxyphenylmethanesulfonanilide is supplied under the trademark "Staticin" by Sharp & Dohme, Inc., Philadelphia.

Results

The particulars of the experiment are presented in Figure 1. There was an unquestionable reduction in the clearance of PSP under the influence of caronamide: the average clearance of PSP in the two control periods, 1 and 2, was 154.68 ml. per minute;* in the transition period, 3, during which caronamide was distributing itself in the body fluids and establishing the physiologic inhibition of PSP excretion, the clearance was 126.16 ml. per minute;* and the average of the three periods, 4, 5, and 6, influenced by caronamide was 23.77 ml. per minute*



		1	2	3	4	5	6
Urine (cc.) per period		154	188	146	148	164	138
Urine (cc.)/min.	= V	10.27	12.53	9.73	9.87	10.53	9.20
PSP in urine (mg./100cc.)	= U	8.21	6.84	7.91	0.87	1.58	1.27
U x V		84.31	85.71	76.96	8.59	16.64	11.68
PSP in plasma (mg./100cc.)	= P	0.53	0.57	0.61	0.52	0.49	0.49
U x V − P		159.07	150.37	126.16	16.52	33.95	23.84

FIG. 1

or approximately $\frac{1}{7}$ of the clearance before the administration of caronamide. It is known that, at plasma concentrations of PSP below 1 mg. per 100 ml., about 80 per cent of the dye is bound to proteins.[14] Since the level of PSP in the plasma throughout the above experiment varied so slightly, the protein-binding may be regarded as having been constant throughout the period of observation. No useful purpose appeared to be served by introducing a protein-binding correction in the calculations of PSP clearances when the object of the

* These clearance figures cannot be interpreted in terms of glomerular filtration rate inasmuch as simultaneous determinations of glomerular filtration rate (mannitol or inulin) and renal plasma flow (PAH) were not done.

experiment was solely to demonstrate a positive effect of caronamide upon the clearance of the dye and not to obtain exact clearance figures for PSP. The plasma concentrations of caronamide that effectively reduced the PSP clearances in this experiment were comparable to those known to inhibit the excretion of penicillin.[6] The results of this experiment have been duplicated in other

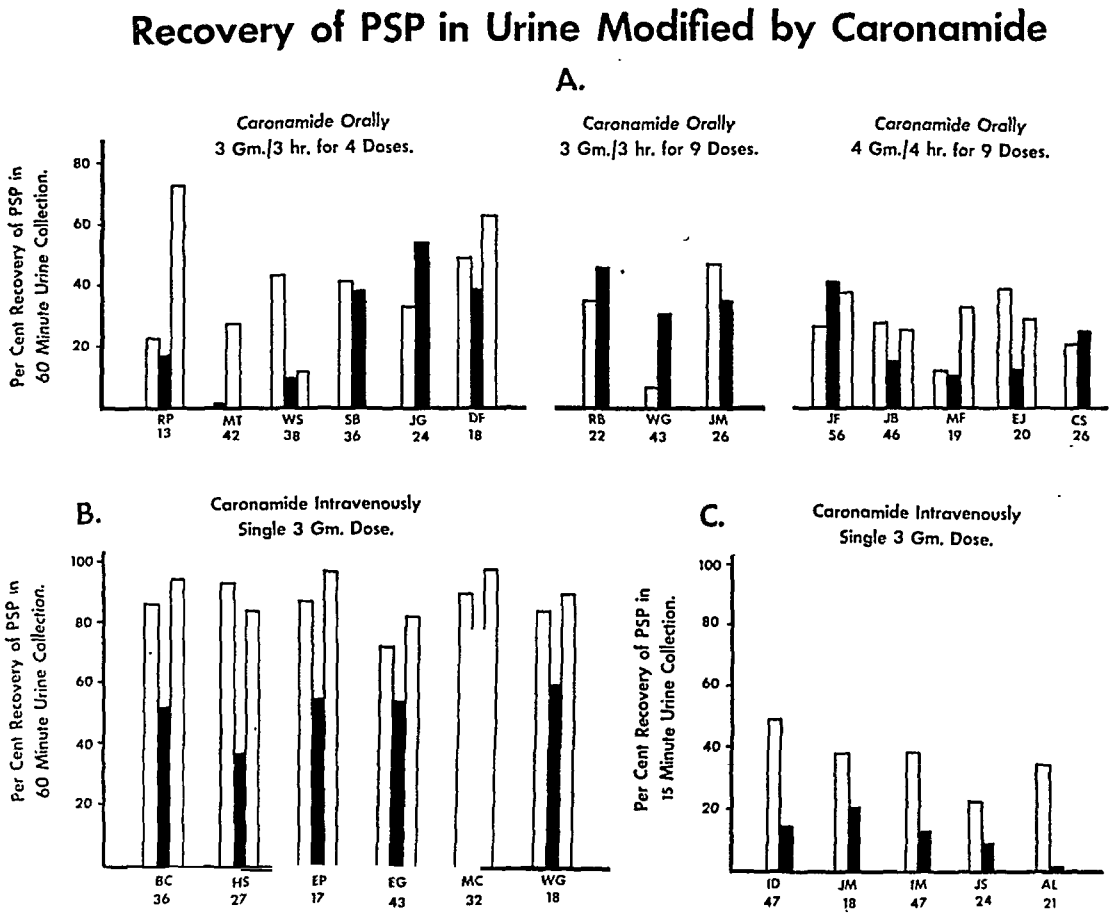


FIG. 2. PSP 6 mg. (1 cc.) was injected intravenously for each test. PSP tests were done on consecutive days at intervals of approximately 24 hours. Solid bars indicate recoveries modified by caronamide. The initials and ages of patients are indicated.

patients and it can be said that caronamide is capable of suppressing the excretion of PSP by the human kidney.

Sources of error

The PSP test as performed in most hospitals gives results that, at best, permit limited interpretation. Failure to measure the dye accurately (measurement is almost always inaccurate unless calibrated syringes are employed), loss of dye during injection, incomplete emptying of the syringe, inability of the patient to empty the bladder completely and use of faded standards may all

contribute to the inaccuracy of the test. These sources of error are familiar to everyone, but the fact that they may completely invalidate the results of the test is sometimes overlooked. PSP test performed without special precautions to obviate these errors are hereinafter referred to as "routine" tests.

SUBSEQUENT EXPERIMENTS

Effect of Oral Administration of Caronamide

Cognizant of the errors that may be introduced during the performance of a PSP test, an effort was made to determine whether the oral administration of caronamide influenced the excretion of the dye sufficiently to be detected by "routine" tests in a hospital ward. Fourteen patients (11 males and 3 females) ranging in age from 13 to 56 years were studied. All of the subjects were ambulatory and the only requirement for inclusion in the study was the ability of the patient to void when requested to do so. The full pattern of experiment was fulfilled in only 7 of the 14 patients (Figure 2A, patients RP, WS, DF, JF, JB, MF, and EJ). In these 7 patients, a control PSP test before the administration of caronamide was compared to one during, and another twenty-four hours after, caronamide therapy. Only one control PSP test was done in the other 7 patients. One milliliter of PSP solution (6 mg.) was employed intravenously for each test and 60-minute collections of urine were made. Caronamide was administered orally in doses that have in the majority of patients provided caronamide plasma-concentrations sufficient to inhibit the excretion of penicillin.[6] In five patients (JG, RB, WG, JF, and CS), the recovery of PSP in the urine was actually greater during the period supposedly modified by caronamide administration than in the control period. In six patients (RP, WS, DF, JB, MF, and EJ), the recovery of PSP following the adminisration of caronamide was less than on the two control days when no caronamide was given. It is noteworthy, however, that in two of these same patients (RP and WS), the results of the PSP tests on control days were widely different; indeed, in the case of patient RP the disagreement was so great as to overshadow completely the difference between one of the control PSP tests and the one possibly modified by caronamide. Similar inconsistencies in the observations made on these 14 patients made it clear that either the PSP test performed in a "routine" manner did not demonstrate a consistent effect of caronamide upon PSP excretion or caronamide administered orally in the doses indicated did not establish caronamide plasma-concentrations sufficiently high to inhibit the tubular excretion of PSP.

Effect of Intravenous Injection of Caronamide

Six additional patients, all of whom were ambulatory and convalescent, were studied. PSP tests were made on three consecutive days; those on the first and third days were control tests and that on the second day was performed following the intravenous administration of 3 Gm. of caronamide. The performance of the PSP tests were standardized in the following ways: (a) One person performed all of the tests, (b) the possible error in the measurement of 1 ml. of PSP solution was minimized by assigning one syringe to each patient and employ-

ing this syringe for each of the three tests performed, (c) a large hourly volume of urine was assured by having each patient drink 300 ml. of water one hour and two hours before each test, and any errors incident to incomplete emptying of the bladder were thereby minimized; and (d) all urine samples were refrigerated until the completion of tests in all 6 patients, and the percentages of PSP recovered were then determined by means of a photoelectric colorimeter. The intravenous administration of caronamide obviated the vagaries of gastrointestinal absorption that may have contributed to the inconclusive results following the oral administration of caronamide. It can be observed in Figure 2B that there was good agreement in the recovery of PSP in the two control tests in each of the 6 patients. Likewise, caronamide caused in all patients a definite reduction in the amount of PSP recovered in the urine. Using the lower value of the two control PSP tests for comparison with the percentages of PSP recovered following administration of caronamide, it was found that the least reduction in PSP clearance was 13 per cent (MC) and the greatest 55 per cent (HS).

At the low plasma concentrations of PSP, estimated to be below 0.1 mg. per 100 ml., that obtain following the injection of 6 mg. of dye, most if not all of the dye is cleared by a combination of glomerular filtration and tubular excretion, but predominantly by tubular excretion, during a single circulation through the kidney.[14] Hence, since PSP is excreted in part by the glomeruli, even though tubular excretion be suppressed to a marked degree, glomerular filtration alone could account for the excretion of most of the injected dye if the blood circulates often enough through the glomeruli. Since the blood passes through the renal circulation many times in the course of an hour, the reduced excretion of PSP by the renal tubules, whether caused by intrinsic renal impairment or pharmacologic inhibition, is rendered less apparent by collecting urine for a period as long as one hour. Clinical experience has established the fractional PSP test[7] as being a more sensitive measure of renal function than the originally described technic[12] and, accordingly, the effect of caronamide on the 15-minute recoveries of PSP was determined.

Five patients, all of whom were ambulatory and convalescent were studied in a manner similar to that described above, the chief exception being that only a single control test was performed prior to testing the PSP excretion following the administration of caronamide. The close agreement of the control tests obtained in the previous group of patients (Figure 2B) seemed to justify the omission of one control test. It may be observed in Figure 2C that there were sharp reductions in PSP recovery in the urine following the intravenous administration of 3 Gm. of caronamide. The least reduction in recovery was 46 per cent of that observed in the control test (patient JM) and the greatest reduction, 100 per cent, observed in patient AL. The greater sensitivity of the 15-minute PSP test in detecting suppressed tubular excretion of the dye is clearly indicated.

DISCUSSION

The sources of error in the "routine" performance of PSP tests have been mentioned above. The results presented in Figure 2A demonstrate clearly that,

even though the tests are done in close time-relationship to each other, the urinary recoveries of PSP in the same patient may vary so widely as to obscure the effect of a drug capable of inhibiting the tubular excretion of PSP. On the other hand, when the PSP test is accurately performed by observing a few precautions, the results on duplicate tests compare favorably. Under these standardized conditions it has been shown that caronamide is capable of suppressing promptly the tubular excretion of PSP. The extent of reduction in the excretion of the dye is made more definite by comparing 15-minute collections of urine rather than hourly collections of urine.

Oral doses of caronamide administered to the group of patients in Figure 2A, 3 Gm. every three hours or 4 Gm. every four hours, have been shown to give, in most patients, plasma concentrations of caronamide between 20 and 40 mg. per 100 ml.[6] Plasma concentrations of this magnitude following intravenous injection are recorded in Figure 1 and the inhibitory effect on PSP clearance is definite. It is probable that the failure to demonstrate a consistent effect of caronamide on the excretion of PSP in the patients receiving oral caronamide was due to faulty technic in the performance of the PSP tests rather than to inadequate dosage of caronamide, but the possibility that adequate plasma concentrations of the caronamide were not attained cannot be ruled out since plasma estimations of the drug were not made. Some patients have been encountered who failed to absorb caronamide in sufficient quantity to give plasma concentrations above 15 mg. per 100 ml. even though 24 Gm. of drug was administered daily, but such patients have been the exception rather than the rule. From the results presented in Figures 1, 2B and 2C, it is clear that caronamide injected intravenously inhibited the excretion of PSP and that the reduction in the clearance of the dye was caused by a temporary and reversible inhibition of a renal tubular transport-mechanism. In Figure 2B it may be observed that, within 24 hours after the discontinuance of caronamide therapy, the PSP clearance returned to normal. It is quite unlikely that the suppression of PSP clearance was caused by actual renal damage by caronamide. This statement is supported by the finding that, in other patients to whom caronamide has been administered for many weeks, the PSP clearance has been shown to return to pretreatment values within 24 hours after the discontinuance of therapy. Similar results have been obtained by other investigators.[10] The only exceptions to this statement have been observed in patients in whom there was pre-existing renal disease and, in consequence, caronamide was not totally excreted within twenty-four hours as it is by persons with normal renal function.

Experiments in 14 patients, as illustrated in Figure 2A, make it appear doubtful whether "routine" PSP tests would be serviceable in indicating the adequacy of caronamide dosage during penicillin therapy. If, on the other hand, reasonable precautions are observed during the performance of the PSP test, it should be a simple and reliable means of determining indirectly that caronamide is present in the plasma in sufficient concentration to inhibit the tubular excretion of penicillin. The recovery of dye in 15-minute collections of urine would appear to be more informative than 60-minute collections. Certainly a PSP test is simpler and more readily available than the facilities for the determination of caronamide

in the plasma;[16] but the latter and more accurate procedure, of course, is to be preferred whenever available. The usefulness of the PSP test as a clinical guide to the effectiveness of caronamide in inhibiting penicillin excretion has been recently confirmed.[10]

CONCLUSIONS

The renal tubular excretion of phenolsulfonphthalein is inhibited by caronamide. This inhibition of excretion is temporary and reversible, the reduction in phenolsulfonphthalein clearance reflecting the pharmacologic action of caronamide and not renal impairment. Phenolsulfonphthalein tests performed with care, particularly when 15-minute recoveries in urine are compared, may be used clinically to indicate indirectly the existence of plasma concentrations of caronamide that are adequate to suppress the tubular excretion of penicillin.

REFERENCES

1. Beyer, K. H.: New concept of competitive inhibition of the renal tubular excretion of penicillin. Science, **105**: 94–95, 1947.
2. Beyer, K. H., Flippin, H. F., Verwey, W. F., and Woodward, R.: The effect of para-aminohippuric acid on plasma concentration of penicillin in man. J. A. M. A., **126**: 1007–1009, 1944.
3. Beyer, K. H., Russo, H. F., Patch, E. A., Tillson, E. K., and Shaner, G.: Certain pharmacologic properties of 4′-carboxyphenylmethanesulfonanilide (caronamide), including its effect on the renal clearance of compounds other than penicillin. J. Pharm. and Exper. Therap., **91**: 272–287, 1947.
4. Boger, W. P., and Baker, R. M.: A comparison of effect of caronamide and benzoic acid or penicillin plasma concentrations. Proc. Soc. Exper. Biol. and Med., **66**: 1–4, 1947.
5. Boger, W. P., Kay, C. F., Eisman, S. H., and Yeoman, E. E.: Caronamide, a compound that inhibits penicillin excretion by the renal tubules, applied to the treatment of subacute bacterial endocarditis. Am. J. M. Sc., **214**: 493–502, 1947.
6. Boger, W. P., Miller, A. K., Tillson, E. K., and Shaner, G. A.: Caronamide: plasma concentrations, urinary recoveries, and dosage. J. Lab. and Clin. Med., **33**: 297–308, 1948.
7. Chapman, E. M., and Halsted, J. A.: Fractional phenolsulphonephthalein test in Bright's disease. Am. J. M. Sc., **196**: 223–232, 1933.
8. Corcoran, A. C.: Personal communication to author (W. P. B.).
9. Crosson, J. W., Boger, W. P., Shaw, C. C., and Miller, A. K.: Caronamide for increasing penicillin plasma concentration in man. J. A. M. A., **134**: 1528–1532, 1947.
10. Hunter, R. B., and Wilson, W. M.: Effect of caronamide on blood-penicillin concentrations in man. Lancet, **255**: 601–604, 1948.
11. Rammelkamp, C. H., and Bradley, S. E.: Excretion of penicillin in man. Proc. Soc. Exper. Biol. and Med., **53**: 30–32, 1943.
12. Rowntree, L. G., and Geraghty, J. T.: The phthalein test: an experimental and clinical study of phenolsulphonphthalein in relation to renal function in health and disease. Arch. Int. Med., **9**: 284–338, 1912.
13. Smith, H. W.: The Physiology of the Kidney. New York: Oxford University Press, pp. 72–91, 1937.
14. Smith, H. W., Goldring, W., and Chasis, H.: The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. J. Clin. Investigation, **17**: 263–278, 1938.
15. Sweet, H. C., Falk, O. P. J., and Agar, Dr.: The effect of caronamide on penicillin therapy. Southern M. J., **41**: 326–334, 1948.
16. Ziegler, C., and Sprague, J. M.: A colorimetric determination of caronamide. J. Lab. and Clin. Med., **33**: 96–100, 1948.

CLINICOPATHOLOGIC CONFERENCE*

C. H. BINFORD, M.D., AND JOHN B. SPRIGGS, M.D.

From the Pathology Service, U. S. Marine Hospital, Baltimore, Maryland

CLINICAL DATA

History. A 33 year old Swedish seaman was admitted to the hospital, unconscious. Four days before, a heavy sea, washing over the deck of his ship, had thrown him against the superstructure and caused a compound fracture of the right tibia. He received medical aid aboard ship from his captain who applied an improvised splint to the right lower leg. About fourteen hours after the injury he became comatose and remained so.

Physical examination. He was well developed and well nourished. There was no evidence of recent head injury. The pupils were dilated, equal and reacted sluggishly to light. His respirations were deep, rapid and sonorous. Rhonchi were audible throughout the chest and there was evidence of bilateral hypostasis. The heart was normal. The blood pressure was 145/86, the temperature 102.4 F., the pulse rate 100 and the respiratory rate 28. There were no abnormal findings on abdominal examination. There was a compound, comminuted fracture of the middle third of the shaft of the right tibia with marked ecchymosis extending over the entire lower leg and into the plantar surface of the foot. No pathologic reflexes were elicited. The Achilles tendon reflexes were greatly diminished. There were no other abnormal neurologic findings.

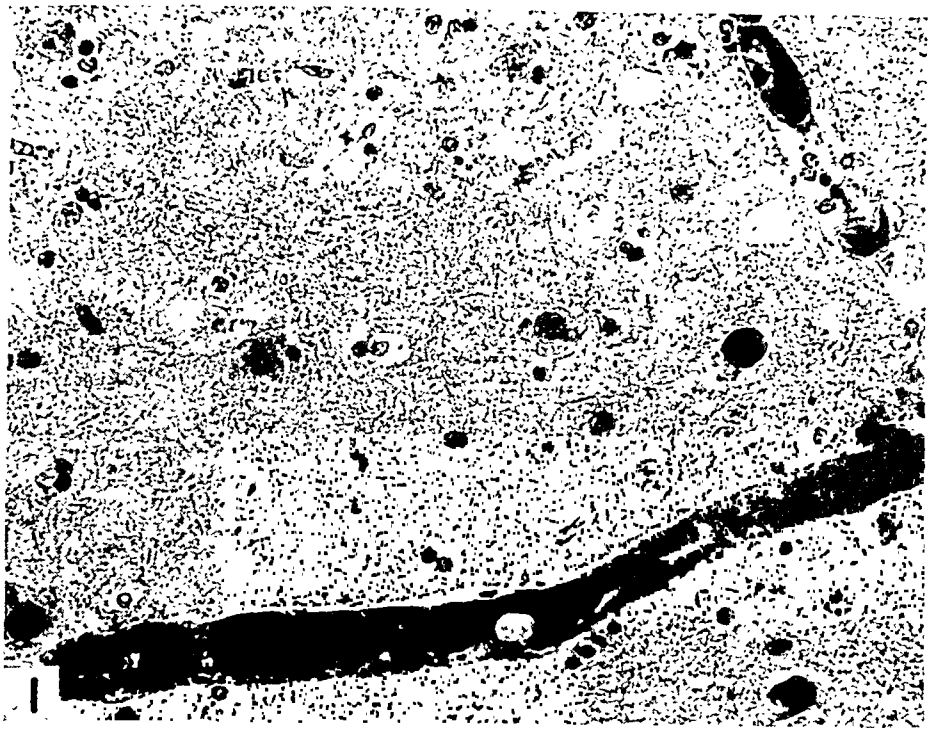
Laboratory findings. Urinalysis revealed one-plus albumen and an occasional granular cast. The Kline and Eagle blood tests were negative. The leukocyte count was 9200 with 84 per cent neutrophils and 16 per cent lymphocytes. The blood sugar was 172 mg., blood urea nitrogen 49.8 mg. and blood creatine 1.1 mg. per 100 ml. The cerebrospinal fluid was clear, its cell count normal, total protein content 37.7 mg., and the Kahn and gum mastic tests negative. Portable x-ray examinations showed the comminuted fracture of the right tibia but did not reveal pulmonary changes.

Course. The patient did not regain consciousness. Spinal puncture revealed normal pressure without evidence of block. The temperature gradually rose to 107 F., and the pulse rate to 160. The blood pressure varied from 140/70 to 158/90. The patient died thirty-six hours after admission.

Discussion

Dr. J. B. Spriggs. "It is significant that the history obtained from the patient's shipmates disclosed that four days before entry he sustained severe injury to his right leg and that he did not lose consciousness until some fourteen hours later. On examination he had a compound, comminuted fracture of the right tibia and he showed no localizing neurologic signs. There was evidence of pulmonary changes which were suspected to represent an early hypostatic pneumonic

* Received for publication, November 29, 1948.



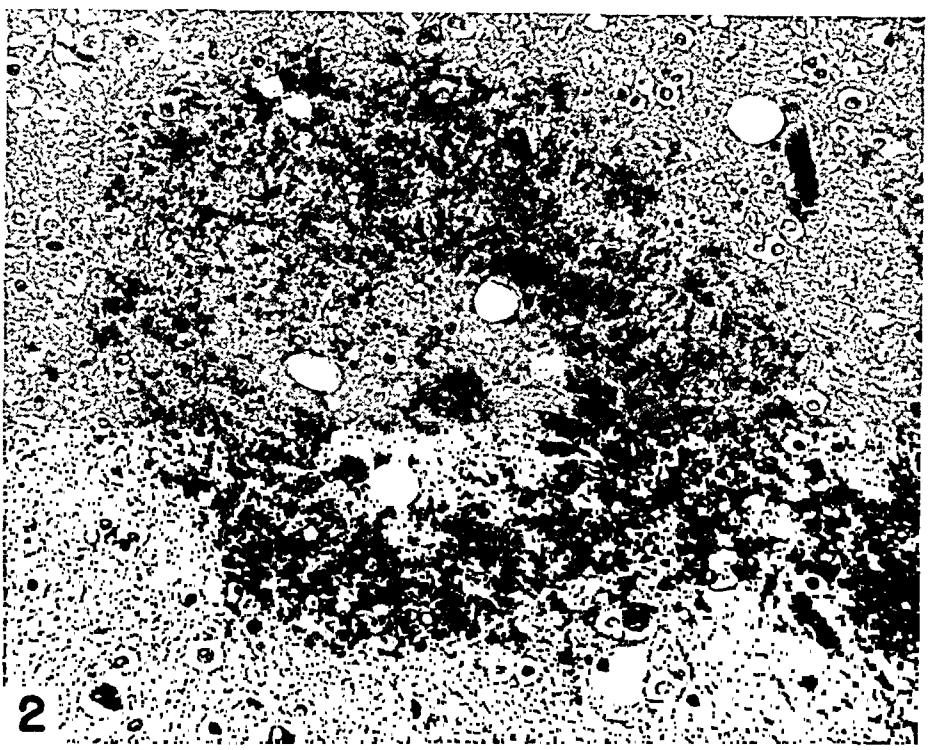


FIG. 1. Frozen section of brain stained with Sudan IV, showing fat-filled capillaries. Green filter. × 400.

FIG. 2. Perivascular hemorrhage and necrosis in section of brain. Romanowsky's stain. × 400.

process. The temperature and the pulse and respiratory rates were moderately elevated. A spinal puncture was negative. The only significant laboratory findings were hyperglycemia and an elevated blood urea nitrogen. Possibly the patient had received intravenous glucose prior to the blood sugar estimation, though no mention of this is made in the protocol.

"He was undoubtedly dehydrated on admission, having been unconscious aboard ship for over three days. Dehydration may partially account for the elevated blood urea nitrogen and for his fever. Despite treatment this patient died thirty-six hours after admission. The only significant findings during this period were the gradual increase in the pulse rate and the steadily rising temperature. In the differential diagnosis one must first consider the possibility of head injury. There was no history of head injury and the patient did not lose consciousness until fourteen hours after his injury. The absence of localizing neurologic signs and the normal cerebrospinal fluid are against significant intracranial damage.

"This case corresponds to a picture which was impressed on me by my professor of pathology, Dr. Carl Weller of the University of Michigan. Dr. Weller repeatedly warned in his lectures of the danger of fat embolism following severe fractures of the long bones. This patient had a severe compound, comminuted fracture of the tibia and there was marked ecchymosis of the lower extremity. It is conceivable that small fat globules liberated into the circulation over a period of time could have produced the picture in this case.

"The patient had pulmonary signs as shown by the rhonchi; he had renal signs as shown by the elevated blood urea nitrogen and he had cerebral signs as manifested by his coma and subsequent death. In most cases of fat embolism symptoms occur twelve to fourteen hours after injury, probably because of the necessity for sufficient accumulation of fat globules in the small arterioles to produce symptoms."

Dr. Emory Moore. "Was the sputum tested for fat?"

Dr. Binford. "No, there is no record of it."

Dr. Moore. "How can you rule out subdural hematoma?"

Dr. Spriggs. "You can't, but I believe most head injuries severe enough to produce subdural hematoma cause bloody spinal fluid. It is possible to have a subdural hematoma without blood in the spinal fluid; however, there should be some xanthochromia of the fluid. The postponement of unconsciousness until fourteen hours after his injury also tends to rule out severe brain damage suffered at the time of injury."

Dr. Emerson Gledhill. "You probably have made the correct diagnosis but how could you rule out epidural hematoma?"

Dr. Spriggs. "There probably would be neurologic changes either in the pupils or in the reflexes unless the hematoma were over a silent area."

Dr. Arthur Koven. "Were there x-ray films of the skull?"

Dr. Spriggs. "No."

Dr. Marion Friedman. "I don't think you have ruled out air embolism."

Dr. Spriggs. "Air embolism is unlikely except in cases where there is a laceration of a large vein such as the subclavian or the internal jugular."

PATHOLOGIC DATA

Dr. C. H. Binford. "By order of Dr. Howard J. Maldeis, Chief Medical Examiner of the State of Maryland, an autopsy was performed by Dr. R. L. Graham, Assistant Medical Examiner. The significant pathologic findings were as follows:

"The right leg showed severe contusion and evidence of fracture of the tibia. Attention was drawn to several petechial hemorrhages in the conjunctiva of the lower lid of each eye and in the skin about each axilla. The prosector pointed out that in the presence of fracture these suggested fat embolism.

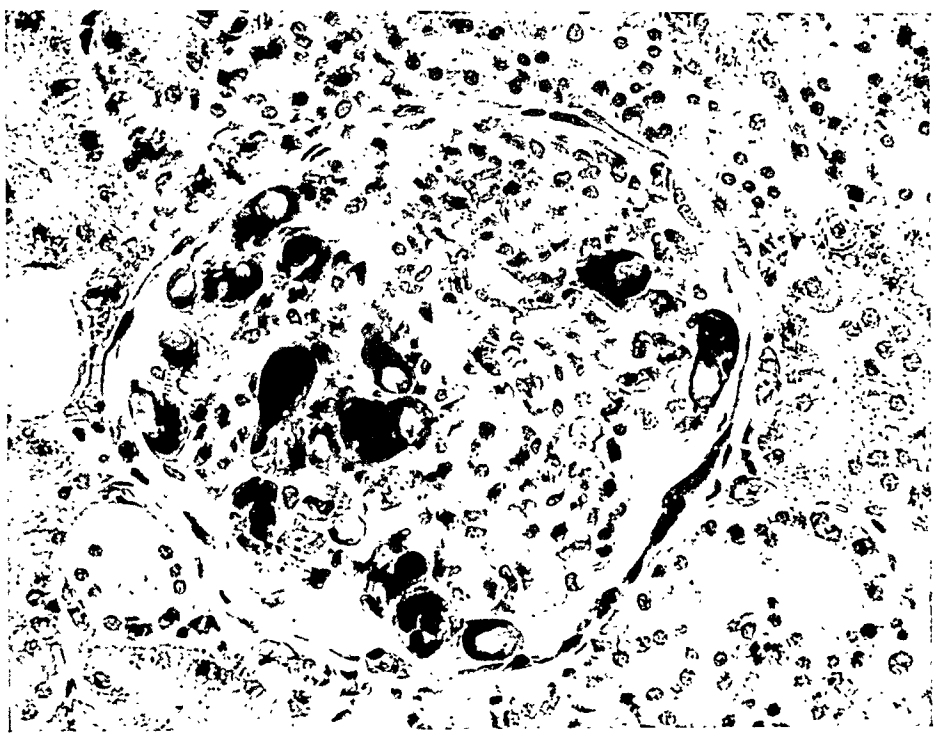


FIG. 3. Frozen section of kidney stained with Sudan IV. Note fat-filled glomerular capillaries. Green filter. × 400.

"The brain showed no evidence of gross hemorrhage. The sulci and convolutions were not particularly abnormal. Cross-sections of the brain showed numerous pinpoint hemorrhages distributed most frequently in the white matter but appearing in the gray matter as well. The pons, medulla and the cerebellum all showed similar lesions. There were no other significant changes.

"No pleural fluid was present. The lower lobe of the right lung showed some patchy consolidation and edema. Section through the upper lobe revealed pinpoint reddish spots. The other viscera revealed no lesion. *Histologic sections* of the brain showed numerous petechial hemorrhages, generally forming collars around minute vessels. Circumscribed areas of vacuolization of the parenchyma were found either directly associated with hemorrhage or unassociated with hemorrhage. Shadows of degenerating cells were seen within these areas. These lesions were more conspicuous in the white matter but were also found in the gray matter.

"Fat stains revealed a number of blood vessels containing material staining red with Sudan IV. Occasionally such fat was seen in the center of an area of hemorrhage.

"The lungs showed patchy exudation of neutrophils associated with considerable congestion and edema. Fat stains revealed septal capillaries and, occasionally, a larger vessel in which neutral fat was demonstrated.

"In the kidneys the glomerular capillaries formed tufts that seemed larger than normal. Within the capillaries many vacuoles were demonstrated but very few red blood cells were seen. Fat stain revealed numerous emboli of lipoid material within the capillaries. The epithelial cells of the convoluted tubules were swollen. The nuclei generally were well preserved but the granular cytoplasm completely obliterated the lumens of most of these tubules.

"In the skin from the axilla there was a small area of hemorrhage in the corium.

"Pathologic diagnosis: Fracture of the right tibia with fat embolism to brain, lungs, kidneys, skin and conjunctivae."

COMMENT

Dr. Binford. "There is an excellent review of fat embolism by Dr. Shields Warren[1] in the recent literature. He reported a group of 100 consecutive cases studied at the Army Institute of Pathology during four years of military mobilization. Ninety-one of these cases followed fractures of one or more of the long bones. The tibia or femur, or both combined, were involved in 82 per cent of the cases associated with fracture. Dr. Warren points out that the essentials for the entry of fat into the circulation are: mobilized fat, veins that are disrupted and patent, and local tissue pressure. It appears that the mobilized fat is still fluid, can be forced into various shapes and may penetrate the finest capillaries. The gross and microscopic pathologic findings in this case are typical of this condition. The axillary and conjunctival hemorrhages were missed on clinical examination but were demonstrated by the Assistant Medical Examiner. Undoubtedly all of us here will look for petechiae when another patient with fracture of a long bone loses consciousness and shows signs of 'hypostatic pneumonia'. No specific diagnostic aids are offered. Neutral fat droplets in sputum or urine might aid in establishing a diagnosis, but I doubt that these tests are positive with sufficient frequency to be of practical assistance. Dr. Spriggs mentioned the fourteen hour interval between injury and loss of consciousness as being consistent with fat embolism. Dr. Warren found a "free interval" in 58 per cent of his cases. Nine per cent, however, died immediately, and 17 per cent had symptoms of fat embolism from the time of injury. This case demonstrates that the autopsy must be carefully performed in order to establish a diagnosis. It was fortunate in this case that the services of a trained Medical Examiner were available. It is possible that the peacetime rarity of diagnosis of this condition is to be explained partly by persistence of the antiquated coroner's system in many of our cities where postmortem examinations are not routinely performed by pathologists."

REFERENCE

1. Warren, Shields: Fat embolism. Am. J. Path., **22**: 69–87, 1946.

EDITORIAL

"Round Cell Infiltration"

Words are merely symbols and, being symbols, have limited values. All the words at one's command do not make the sunset they are intended to describe. A Beethoven symphony cannot be reconstructed from the pages of a critic's panegyric. In similar fashion, a protocol is merely a pale but necessary substitute for the body, its organs, and the microscopic sections made from them. Hence, accuracy of description in pathologic anatomy is the sole means of transforming a written report from mere words into intelligible symbols. When elements in the anatomic picture are not readily identified, the need for that accuracy is greater than ever. The use of a stock phrase as a surrogate, therefore, is especially regrettable.

"Round cell infiltration" is such a phrase, used by the high and low. The fifth edition of Ziegler's "Lehrbuch", published in 1887, for example, is liberally sprinkled with "round cells" and "little round cells". Much of the confusion about the criteria of Riedel's struma, first described nine years later, rests on the brief note that the scarred tissues were infiltrated by "round cells". It is common experience at any medical meeting to hear glib mention of "round cell infiltration" pass without challenge.

Recently a letter was sent to several academic and clinical pathologists asking whether they employed the phrase "round cell infiltration" and, if so, what cellular elements make up the infiltrate. Almost to a man they denied using such a term and strongly condemned it, adding that, as they understood its use by others, it referred to lymphocytes, and perhaps also to plasma cells. This identification apparently is not universal; Wahlberg, in his 1939 study of thyroid disease in Finland, wrote "round cell infiltration of granulocytic type".

If "round cells" can be recognized as monocytes, lymphoid cells, or plasma cells, they should be properly named. If identification is not made, the cells in question ought to be described as well as possible to assist the reader. The phrase "round cell infiltration" in any manuscript, especially in one submitted to a journal devoted to pathology should be the signal for its prompt return to the author with a note of explanation. Such action on the part of editors, aided by voluntary efforts of all pathologists, clinical as well as academic, not to use or recognize the term, should hasten that blessed event, the demise of "round cell infiltration".

Fort Wayne, Indiana S. M. Rabson

SELECTED ABSTRACTS

Experimentally Induced Changes in the Prothrombin Level of
of Vitamin K Deficiency to the Intensity of Dicumarol Acti
Vitamin A Intake; With A Simplified Method for Vitamin
AND MARIO STEFANINI. J. Biol. Chem., **175**: 945–952, 1948.

The authors describe a simple vitamin K-free diet which
thrombinemia in newly hatched chicks (within ten days). Th
vitamin K assay.

It was noted that 1.5 to 2 gammas of 2-methyl-1,4-naphthoq
2.5 gammas of natural vitamin K_1 per day was required to mai
of chicks during the first ten days of life. The injection o
deficient chicks caused the prothrombin activity to return, s
sively more rapidly, gradually becoming normal after four h
beyond that minimally effective, did not influence the speed
activity.

The susceptibility of chicks to the action of Dicumarol w
vitamin K from the diet. The addition of vitamin K coun
binemia. No difference was noted between the efficacy of
nadione. Excessive intake of vitamin A, which causes hy
had no effect on the prothrombin level of chicks receiving vitam

Cleveland

Vitamin B_{12} Therapy in Pernicious Anemia. B. E. HALL A
Staff Meet. Mayo Clinic, **23**: 585–595, 1948.

Eleven patients with pernicious anemia in relapse were tr
jections of vitamin B_{12} and were followed for periods of on
ranged from 1 microgram daily to 25 micrograms once a week
maximal reticulocyte response, when compared to liver extra
reaction was suboptimal. The red blood cells rose to normal
tients who were followed longer than two months. Bone ma
nine hours or longer after administration of vitamin B_{12} showe
megaloblastic picture, with conversion to the normoblastic
of vitamin B_{12} there was improvement in the general well-bei
mental alertness and appetite. The glossitis disappeared wit
and signs of peripheral neuritis and subacute combined deg
relieved remarkably soon after institution of therapy.

This reviewer has had the opportunity to study patients wit
with vitamin B_{12} and is able to confirm the laboratory and cli
as reported by the authors. The therapeutic dose by the int
be between 0.75 and 1.0 micrograms per day.

Brooklyn, New York

BOOK REVIEWS

Hematology. By CYRUS C. STURGIS, M.D., Professor of Internal Medicine, Chairman of the Department of Internal Medicine, University of Michigan Medical School and Director of the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan. 915 pp., 72 figs., 44 tables. $12.50. *Springfield, Ill.:* Charles C Thomas, 1948.

This large but compact volume by Sturgis is an excellent presentation by a unique personalized approach of seasoned knowledge in the field of hematology, based upon an extensive well balanced general clinical experience. The anemias are clearly presented and discussed in their proper sequence, beginning with the common types of iron deficiency anemia and closing with the less frequently observed anemic states such as elliptocytosis, Mediterranean anemia and erythroblastosis fetalis. The hemorrhagic and purpuric disorders are discussed in relation to the chemical constituents that enter into the coagulation mechanism as well as those clinical conditions associated with platelet deficiency and vascular defects. Some of the hemorrhagic syndromes are presented in a clinical fashion and the diagnosis and therapy are enhanced by the author's personal experience. Disorders associated with quantitative and qualitative changes in the leukocytes are well covered in the chapters that include infectious diseases, leukemias and variants in this group (chloroma, multiple myeloma) and clinical states associated with leukopenia, agranulocytosis and infectious mononucleosis. The subject of malignant lymphoma is well written and important diagnostic points are emphasized. The chapter on polycythemia is concise and treatment is embraced in detail. The section on blood transfusions and blood substitutes contains a mass of useful and well-documented data. It is regrettable that the author did not avail himself of much of the excellent work on bone marrow that has been published in monograph form and in articles in both the foreign and English literature on sternal puncture. Such additional data would aid materially in the clinical interpretation of sternal puncture and would be of added assistance in the diagnosis and treatment of blood dyscrasias.

The presentation of historical aspects of hematology is useful and original, and the placing of references at the bottom of each page makes it easier for the reader to find them for consultation. The extensive list of 1830 references arranged alphabetically according to author has been chosen with great care. Diagnosis and therapy are the main themes of this book. Morphologic hematology is subordinate and accordingly serves its purpose primarily in a confirmatory manner. The reviewer agrees with the author that the morphologic evaluation of hematology is an adjunct to diagnosis and best serves its purposes when correlated with the more important clinical findings. On the other hand the possibility must never be overlooked that in certain cases the study of the blood and bone marrow alone may lead to the proper diagnosis, treatment and prognosis.

The photographs are not up to standard in their color value and thus do not serve as a helpful guide in the morphologic identification of blood cells as observed in some blood diseases. The few typographic errors and the more recent advances in hematology can await the next edition of this book for their correction and inclusion.

The book is well written, easy to read and authoritative, and can be highly recommended.

Chicago LOUIS R. LIMARZI

The Liver and Its Diseases. By H. P. HIMSWORTH, M.D., F.R.C.P., Professor of Medicine in the University of London; Director of the Medical Unit, University College Hospital, London. 204 pp., 69 illus. $5.00. Cambridge, Mass.: Harvard University Press, 1947.

In this book, primarily experimental-pathologic and morphologic observations are utilized to discuss the hepatic diseases. As expected from one with a personality as dynamic as the author and from one who has made excellent contributions previously, the book represents a fascinating and challenging treatise. Despite some obvious over-simpli-

fications, the reasoning is interesting and stimulates further thought and work. It is surprising how wide a field of hepatic pathology is covered or at least illuminated in this small book. In the introduction, zonal and massive hepatic necrosis are differentiated, the former often resulting in complete regeneration, the latter leading to collapse of the parenchyma. Fibrosis of the liver (a term preferred over cirrhosis) occurs in two forms: one follows massive necrosis (postnecrotic cirrhosis) and the other, a diffuse form (like Laennec's cirrhosis), results from repeated attacks of zonal necrosis, prolonged (usually fatty) infiltration of the parenchyma or biliary obstruction.

The thesis is presented that the outcome of any injury of the liver is less determined by the nature of the injurious agent than by the extent of the injury and reactions inherent to the liver itself. Main emphasis is laid on circulatory and nutritional disorders. The former, which are considered to be caused by swelling of the liver cells, lead to either massive or central necrosis. Two types of experimental dietary hepatic injury are differentiated; one caused by cystine deficiency, leading to massive hepatic necrosis and the other associated with fatty infiltration, causing diffuse hepatic fibrosis. The importance of conditioned nutritional deficiency of the liver in well established forms of toxic liver damage is emphasized. From the experimental pathologic observations, a tentative classification of human liver diseases is given, illustrated by a brief survey of their clinical manifestations. In this chapter, original clinical observations are presented. The clinical manifestations are thought to fall into sequences which correspond to the pathologic sequences observed in human beings and experimental animals. Their knowledge is supposed to permit recognition of the still treatable initial lesions of liver diseases and to direct attention to further critical investigations. The discussion is in an excellent, forceful and clear style and well documented with illustrations, to a great part, photomicrographs. Even if all points made cannot be accepted without hesitation, the book can be highly recommended to every clinical pathologist who desires to acquaint himself with the modern trends of hepatic pathology.

Chicago HANS POPPER

Urine and Urinalysis. Ed. 3. By LOUIS GERSHENFELD, B.Sc., P.D., PH.M., D.Sc., Professor of Bacteriology and Hygiene and Director of the Bacteriological and Clinical Chemistry Laboratories at the Philadelphia College of Pharmacy and Science. 347 pp., 42 figs. $5.00. New York: Romaine Pierson Publishers, Inc., 1948.

Centuries ago the visiting physician would examine the patient's urine in a special glass receptacle for color, appearance, odor, taste and quantity. From this he made deductions as to the underlying disease processes. Thus, many outstanding names in medical literature are associated with the study of the urine. Matthew Dodson (1776) of England directed attention to the sweetness of the urine and the blood serum in diabetes and claimed that it was due to the presence of sugar. Johann P. Frank (1791) employed the yeast test for the detection of sugar in diabetic urine. Kruikshank in 1800 was one of the first who employed a boiling and nitric acid test for the presence of albumin in the urine. William Prout (1850), an English chemist, was the first who employed litmus paper in testing the reaction of urine, and H. von Fehling determined quantitatively the presence of sugar in the urine. One can list a large number of other names, such as Bowman, Berzelius, Bence-Jones and Ludwig, and a host of anatomists and physiologists who have studied the structure and function of the kidney and the alterations in the urine accompanying these structural and functional changes. In more recent times, the urine has been studied by clinicians for the examination of crystals associated with sulfonamide therapy, concentration-dilution tests, various kidney function tests, and hormonal pregnancy tests.

Dr. Gershenfeld has revised his previous two editions on "Urine and Urinalysis" and has added newer methods and improved technics for the study of the urine. The fact that his book in a few years has reached three editions is ample proof that there is a need for better understanding of the methods and interpretation of urine findings. This book is recommended to those employed in the examination of the urine as well as the clinician

who will interpret the results of the examination. Above all, Dr. Gershenfeld's book is an answer to some medical students and some members of the hospital house staff who consider that it is beneath their dignity to examine the urine of their patients. The illustrious men of medical history who examined the urine of their patients and who thereby contributed to scientific knowledge and improved our clinical diagnostic ability may well be remembered when we examine the urinary specimen.

Chicago S. A. LEVINSON

The Spleen and Hypersplenism. By WILLIAM DAMESHEK, M.D., AND SOLOMON ESTREN, M.D. 55 pp., 55 charts. $4.75. New York: Grune and Stratton, 1948.

Dameshek has been for a number of years an advocate of hypersplenism, a hypothesis of hormonal depressive action of splenomegaly on bone marrow. The monograph is a reprint of an exhibit presented at the 100th Anniversary Convention of the American Medical Association in Atlantic City in June, 1947. It consists of 55 charts dealing with anatomy, histology, and physiology of the spleen and splenomegaly, the role of the spleen in hemolytic anemia, in idiopathic thrombocytopenic purpura, in cytopenia, neutropenia, and pancytopenia. Other charts cover problems of pathogenesis, diagnosis and treatment.

The presentation is critical and includes both sides of the argument. The authors deserve commendation for having made available a permanent record of their excellent exhibit. It can be recommended as a well organized and didactically useful summary of the present day knowledge of splenomegaly. Numbering of pages and a subject index would have facilitated the use of the book.

Chicago I. DAVIDSOHN

Outline of Histology. Ed. 2. By MARGARET M. HOSKINS, PH.D., AND GERRIT BEVELANDER, PH.D., Departments of Histology, College of Dentistry and The Graduate School of Arts and Science, New York University. 292 pp., 56 figs., 2 plates. $3.50. St. Louis: The C. V. Mosby Company, 1948.

This short outline, intended for dental students, is not complete enough for medical students, or for physicians who seldom refer to histology books, and then only for complete and authoritative information.

The respiratory tract and the liver are each described in only a page and a half. The descriptions frequently consist of definitions of various types of cells and of histologic terms. Space cannot be given to finer histologic details or discussions of embryogenesis or function of the cells described. Without outside reading even from a standard text, it would seem that a student using this outline would find histology an uninteresting subject. There is no index.

Dearborn, Michigan H. J. LINN

Cancer of the Esophagus and Gastric Cardia. Edited by GEORGE T. PACK, B.S., M.D., Clinical Professor of Surgery, New York Medical College; Attending Surgeon, The Memorial Hospital for Cancer and Allied Diseases, New York, New York. 192 pp., 25 illus. $5.00. St. Louis: The C. V. Mosby Company, 1949.

This volume is a symposium embracing all the articles in the June, 1948 issue of the journal, *Surgery*. Unlike a monograph it does not include all aspects of the subject since certain fundamental facts concerning anatomy, physiology and pathology are lacking. For this reason the title is likely to be misleading to one who is unable to review the book prior to purchase. For the surgeon interested in this subject it does present technical achievements not heretofore emphasized in the literature. Outstanding is Dr. Maier's review of pre- and postoperative care, and Dr. Sweet's analysis of over 200 cases requiring esophageal resection. The illustrations are good.

Eloise, Michigan DARRELL A. CAMPBELL

Essentials of Pathology. Ed. 3. By LAWRENCE W. SMITH, M.D., formerly Professor of Pathology, AND EDWIN S. GAULT, M.D., Associate Professor of Pathology and Bacteriology, Temple University School of Medicine, Philadelphia, Pa. 763 pp., 740 illus. $12.00. Philadelphia: The Blakiston Company, 1948.

There is no more suitable text available to the medical student than this interesting, informative and correlative introduction to the essentials of pathology. Students will be enabled to learn less painfully because of the excellent presentation of the case histories, textual material and illustrations, all of which have been improved over previous editions. The book also offers a correlation of facts which aids in the retention of otherwise isolated knowledge. In the main, the text is unusually clear and concise, but there are some confusing passages, such as the description of the mechanism of jaundice on page 460 and the all-too-brief description of lower nephron nephrosis.

Because of the limitations imposed by the authors, the book has neither the extent nor detail to be of much value to the pathologist. It is primarily intended for the medical student but it also has a place of value in the hospital library for interns and residents.

Eloise, Michigan J. L. CHASON

Manual of Clinical Laboratory Methods. Ed. 4. By OPAL E. HEPLER, PH.D., M.D., Associate Professor of Pathology, Northwestern University Medical School, Director of the Clinical Laboratories of the Montgomery Ward Clinics and Passavant Memorial Hospital, Consultant in Clinical Pathology at Children's Memorial Hospital, Chicago, Illinois. 395 pp., 36 figs., 8 full color plates, 84 tables. Springfield, Ill.: Charles C Thomas, 1949.

Modern clinical pathology has become so complex that no one person can hope to become adept at all procedures. This book was written to furnish explicit and detailed instructions for each laboratory test in the interest of accuracy, since accuracy in clinical pathology means accuracy of the individual laboratory technician.

The previous three editions were printed in planograph form. This fourth edition is the first to be published in book form and to contain colored plates illustrating morphology of blood cells and malarial parasites.

The text is written in outline form with numbered headings and subheadings. The pages have double columns. The directions are chronologic, concise and easy to follow. Each test contains a brief section devoted to general principles of the test, interpretation, and sources of error. The clinical correlation is purposely not discussed in detail, and for such information the student will have to consult a textbook on clinical pathology. In addition to the usual chapters to be found in a manual on laboratory methods, there are special sections devoted to electrocardiography, allergy extracts, liver function tests and mycology.

Such a compact and well-written manual has few detectable flaws. Criticism may be made of the use of the term "milligrams per cent" instead of the more precise term "milligrams per 100 milliliters". Likewise, it would have been desirable to use "milliliter" in place of the less exact "cubic centimeter" as a unit measure of liquid or gas. On page 236 regarding preparation of the standard Kahn antigen (IV, A, 3) the author has not followed Dr. Kahn's original instructions in measuring "1.2 cc. of antigen", and in mixing saline and antigen "6 times". The original directions call for 1 ml. of antigen and rapid mixing, twelve times.

The arrangement and type are attractive.

The manual is well recommended for technicians. As stated, those who use it should obtain information on clinical correlations from text books on clinical pathology.

Eloise, Michigan LAURIN J. KAASA

NEWS AND NOTICES

Letters to the Editor

December 14, 1948

To the Editor:

The purpose of this letter is to correct certain assertions made in the article by H. Wallerstein and S. S. Brodie, "The Efficiency of Blood Substitution", which appeared in the American Journal of Clinical Pathology, volume 18, pages 857 to 866, November, 1948.

In their conclusions, these authors claim that they have proved that "the sagittal sinus or umbilical vein technics are approximately 33 per cent more efficient than the radial artery route". As a matter of fact, the article does not deal with the different technics of exchange transfusion but merely provides mathematical formulae for the percentage of replacement when blood is introduced into and withdrawn from the body simultaneously. The formula given for continuous replacement agrees with the formula previously published by us, while the formula for intermittent replacement agrees with the formula of the Dutch physician, Soeter.

Wallerstein and Brodie do show that if the transfusion is started by introducing 50 ml. of blood and the baby is maintained in this state of plethora throughout the procedure, the replacement is 33 per cent less efficient, using the same 500 ml. volume of donor's blood, than if one starts the exchange transfusion by withdrawing 50 ml. of blood and the baby is maintained in this condition of reduced blood volume throughout the procedure. This calculation is valid *regardless of the route of transfusion*, so that the conclusion that the radial artery method is 33 per cent less efficient is totally unwarranted. We maintain, regardless of the route used, that to keep the baby in the state of plethora during a long procedure is far safer than in a state bordering on shock, especially when the baby is anemic to begin with. Finally, since we routinely use 1000 ml. instead of 500 ml. of blood for exchange transfusions, we accomplish a 98 per cent replacement instead of only 90 per cent in all our cases.

64 Rutland Rd., A. S. Wiener
1672 Ocean Ave. I. B. Wexler
Brooklyn, N. Y.

January 11, 1949

To the Editor:

In their criticism of the conclusions drawn in the article, "The Efficiency of Blood Substitution", Dr. Wiener and Dr. Wexler disagree with the opinion that substitution performed through the route of either the umbilical vein or the sagittal sinus is more efficient than through the radial artery. It is stated in the body of that paper that the radial artery method can best be used for the continuous drop-by-drop substitution, while the other two routes lend themselves easily to intermittent withdrawals and replacements by syringe. The use of a syringe makes it possible to alter appreciably the total blood volume in each replacement step, thus mathematically affecting the percentage of substitution. Thus, if one bleeds the patient of one half of his blood volume and replaces an equal amount, and then repeats the step twice again, it will be found that an 88 per cent substitution will have been reached, using one and one-half volumes of donor blood. If steps employing one-fourth volumes are used, one and one-half volumes of donor blood will effect only 82 per cent substitution. The smaller the amount used, the longer the procedure must be continued, and the more donor blood must be used in order to achieve the same results. With the radial artery route, controlled large step replacements are almost impossible.

We disagree also in the need for the "state of plethora". We routinely bleed even anemic infants of one-sixth to one-fourth of their volume at the beginning of the procedure, and with infants who show no anemia we have removed as much as one-half volume before introducing

new blood. We have never encountered either shock or impending circulatory embarrassment. On the other hand, the use of 1000 ml. of donor's blood, containing at least 240 ml. of citric acid-dextrose mixture, does appear to carry some risk to the infant. At a recent meeting of the New York Pathological Society, Dr. P. Rosenblatt presented 4 autopsy reports of infants so treated, and pointed out that the very rapid infusion of such large quantities of citrate might have contributed to the fatal outcome. These infants all showed severe liver necrosis at autopsy. We have not yet experienced a single such instance. In 51 substitutions there have been 7 deaths, all occurring either during or immediately after the substitution. All 7 showed severe kernicterus at autopsy, indicating that this complication was present before treatment was begun. Not a single infant, who was not beyond aid when the procedure was begun, as proven by postmortem examinations, failed to survive and progress normally. Those infants who are now between one and four years of age show no neurologic sequelae. The others are still too young for conclusive findings.

We hold to the belief that if the length of time required for the blood substitution can be shortened, and the amount of donor's blood used can be reduced, better results will be obtained.

We wish to thank Drs. Wiener and Wexler for commenting on this paper.

Jewish Memorial Hospital H. WALLERSTEIN
New York, New York S. S. BRODIE

MICHIGAN PATHOLOGICAL SOCIETY

At the annual meeting of the Michigan Pathological Society on December 11, 1948, the following officers were elected for 1949: President, D. H. Kaump, Detroit; President-Elect, R. E. Olsen, Pontiac; Secretary-Treasurer, W. A. Stryker, Wyandotte; Councilors, A. A. Humphrey, Battle Creek, and S. E. Gould, Eloise.

CHICAGO MEDICAL SCHOOL

The Chicago Medical School was granted full approval by the Council on Medical Education and Hospitals of the American Medical Association. This approval represents a culmination of many years of labor on the part of Dean John J. Sheinin, under whose leadership the college has established an acceptable program of medical education.

BLOOD BANK COMMITTEE

Dr. O. A. Brines, President of the American Society of Clinical Pathologists, has appointed a Blood Bank Committee consisting of the following members:

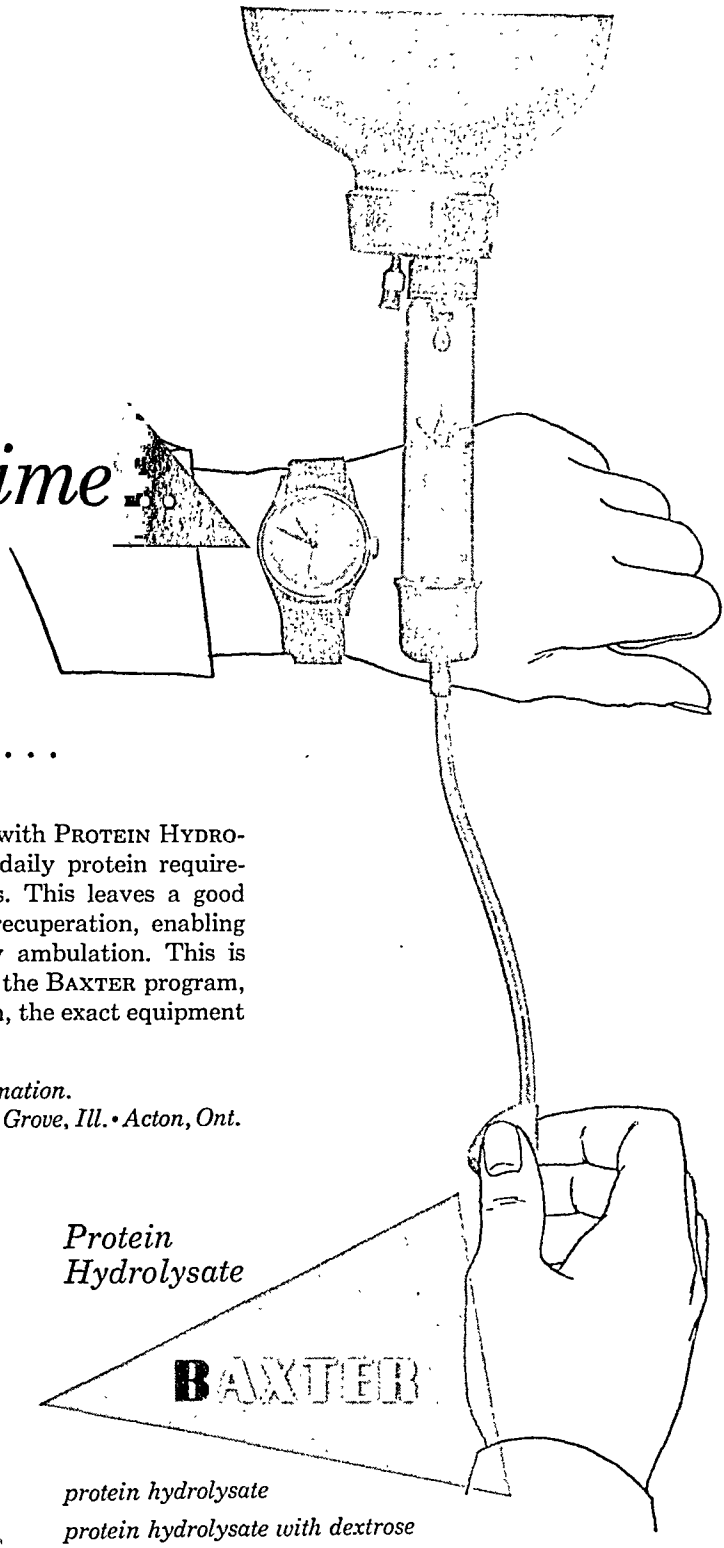
Dr. Donald H. Kaump, Chairman, Detroit; Dr. Lucien Y. Dyrenforth, Jacksonville, Fla.; Dr. Frank W. Hartman, Detroit; Dr. Leonard W. Larson, Bismarck, N. D., and Dr. Ralph G. Stillman, New York.

The purpose of this committee is to act as a clearing house for blood banking information and problems which affect the members of this Society, and to maintain a close contact between the existing agencies and organizations connected with the establishment and operation of blood banks throughout the country.

NOTICE TO DIPLOMATES, AMERICAN BOARD OF PATHOLOGY

Diplomates of the American Board of Pathology are requested to report any inaccuracy in their names or addresses, as listed is the February 1949 issue of the Journal, to Dr. Robert A. Moore, Secretary, American Board of Pathology, Washington University School of Medicine, St. Louis 10, Mo.

short
infusion time



MEANS

more rest time...

earlier ambulation...

The high rate of infusion possible with PROTEIN HYDROLYSATE, BAXTER, provides total daily protein requirements in from two to four hours. This leaves a good portion of the day for rest and recuperation, enabling the patient to benefit from early ambulation. This is another example of the benefits of the BAXTER program, which provides the specific solution, the exact equipment for any parenteral requirement.

WRITE for booklet giving full information.
BAXTER LABORATORIES, Morton Grove, Ill. • Acton, Ont.



Protein Hydrolysate

BAXTER

protein hydrolysate
protein hydrolysate with dextrose
protein hydrolysate with dextrose and alcohol

Available only in the 37 states east of the Rockies (except El Paso, Texas) through

AMERICAN HOSPITAL SUPPLY CORPORATION
General Offices: Evanston, Illinois

(In writing to advertisers, please mention the Journal—it helps.)

NEW • COMPLETE • CLINICAL

Pharmacologic Principles of Medical Practice

BY

JOHN C. KRANTZ, Jr., Ph.D.
and C. JELLEFF CARR, Ph.D.

The title of this new text gives the key to the approach of the subject matter. "Principles" explain what to do and why, and "Practice" how to do it and when.

With these points in mind, the authors have emphasized the clinical application of the action of drugs. The subject matter is arranged according to the physiologic systems of the body. It includes a synopsis of the physiologic and biochemical functioning of these systems as a basis for the understanding and interpretation of drug action.

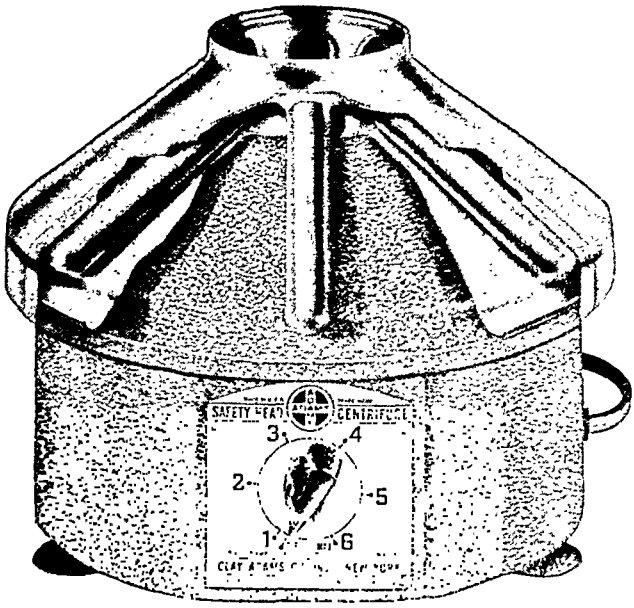
Drugs in everyday use have been selected for discussion, with no attempt made to embrace all the drugs used in treatment. The bibliography is a selective one featuring review monographs, original research, and recent journal publications.

The subject matter is introduced by an interesting and readable account of the scope of pharmacology and its development as a medical science. In this connection, the book contains twenty-four portraits of outstanding figures in pharmacology.

The sections on clinical aspects have been reviewed and criticized by leading men in their respective fields. The information is up to date and embodies the accepted pharmacotherapy.

995 pp. • 94 figs. • 24 portraits • $10.00

THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves. Baltimore 2, Maryland



Now Available!

ADAMS SAFETY-HEAD CENTRIFUGE

A compact, lightweight unit that can be moved readily from place to place in the laboratory, and will fit in a small refrigerator when centrifuging in the cold is required.

Utilizes the angle principle by suspending the tubes at a fixed 52° angle, thus achieving a faster rate of sedimentation and more complete sedimentation with finely dispersed particles.

Accommodates 6 15-ml. tubes; can be used for micro and semi-micro work by substituting the proper shields and tubes.

FEATURES

Head is made of a special aluminum alloy, precision-machined for accurate balance.

Housing is cast aluminum, attractively finished in gray hammertone.

Rheostat provides an "off" position and allows for continuous speed control.

Rubber suction feet absorb all vibration and keep the centrifuge from creeping, even on a glass surface.

Maximum speed when fully loaded is 4000 RPM on AC, 4500 RPM on DC.

CT-1002 Adams Safety-Head Centrifuge for 6 15-ml. tubes; complete with head, 6 round-bottom brass shields, 3 each graduated and ungraduated 12-ml. heavy duty glass tubes, underguard, safety cap; for 110 volt AC and DC. . **each $72.00**

220 volt model supplied at additional cost of $3.00

Write for Circular describing our complete line of centrifuges

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 8, ILL.

(In writing to advertisers, please mention the Journal—it helps.)

"This book is to this reviewer's knowledge the most comprehensive, most reliable and, for practical purposes, best balanced presentation available for the use of the undergraduate student, the general practitioner and the specialist.
"If it is used as much as it deserves, it will represent one of the most realistic contributions of the last decade to the fight against cancer."
ARCHIVES OF PATHOLOGY

CANCER

Diagnosis, Treatment and Prognosis

By

LAUREN V. ACKERMAN, M.D., Pathologist to the Ellis State Cancer Hospital; Assistant Professor of Pathology, Washington University School of Medicine, St. Louis.

JUAN A. DEL REGATO, M.D., Radiotherapis to the Ellis Fischel State Cancer Hospita formerly, Assistant to the Radium Institute the University of Paris.

115 Pages 745 Illustrations and 42 Color Reproductions PRICE, $20.00

FOR GENERAL PRACTITIONERS AND SPECIALISTS

Here is a remarkable and prodigious book—written by two eminently qualified men—one a pathologist, and the other a radiologist, containing so vast an amount of information on Cancer as to quicken the hearts of men in medicine.

PART I is devoted to subjects of general interest and application in the field of malignant neoplasms. PART II discusses Cancer of the various systems and organs of the body, with indications for surgery, radiotherapy, roentgentherapy and curietherapy in practically every instance.

"It is recommended to general practitioners and those who see cancer but do not treat it because it aids in early recognition, and because it tells what can be accomplished with present methods of therapy, thereby removing the entirely hopeless attitude which is still too prevalent."
ANNALS OF INTERNAL MEDICINE

"The specialist in any field will find in here a well planned discussion of cancer in his own department. Also, without much effort he may become familiar with the diagnoses and the therapeutic problems that arise in other fields. It is a good book."
QUARTERLY REVIEW OF SURGERY

----------ORDER FORM-----

The C. V. Mosby Company
3207 Washington Blvd.
St. Louis 3, Missouri

AJCM 4-48

Please send me Ackerman-Regato's CANCER. The price is $20.00

☐ Enclosed find check. ☐ Charge my account.

Name_____

Address_____

(In writing to advertisers, please mention the Journal—it helps.)



BACK of the year-round economy you obtain with PYREX brand laboratory glassware is Corning's long interest in the field of public health. For many years, Corning researchers have done a great deal of development work on glassware to serve your increasingly important requirements.

PYREX brand culture tubes, pipettes, petri dishes, milk-dilution bottles, culture flasks and bacteria filters, for example, are made to exacting standards of chemical glass No. 774. It possesses properties which provide maximum mechanical strength, heat resistance and chemical stability. This means longer service life, greater economy.

Consult your laboratory dealer now. He stocks PYREX brand laboratory ware for you and can give you prompt service.

CORNING means research in GLASS

PYREX T.M. REG. U.S. PAT. OFF.

Stocked by Leading Laboratory Supply Houses

CORNING GLASS WORKS • CORNING, N. Y.

LABORATORY GLASSWARE

TECHNICAL PRODUCTS DIVISION: LABORATORY GLASSWARE • GAUGE GLASSES • GLASS PIPE
LIGHTINGWARE • SIGNALWARE • OPTICAL GLASS • GLASS COMPONENTS

(In writing to advertisers, please mention the Journal—it helps.)

Written to bridge the physiological gaps between the concept of the disease held by the internist and that held by the psychiatrist

FUNCTIONAL CARDIOVASCULAR DISEASE

By MEYER FRIEDMAN, M.D.

The Harold Brunn Institute for Cardiovascular Research, Mt. Zion Hospital, San Francisco

Many books have been written on disorders of the cardiovascular system, but there are few on functional aberrations. Since the physician sees the latter as well as the organic derangements, a complete understanding of the physiologic nature of the disease becomes necessary.

Doctor Friedman has made an important contribution in clarifying the terminology for a disease variously called "Irritable Heart," "Soldier's Heart" and "Disordered Action of the Heart." He has applied exhaustive physiological methods to explain the occurrences of various phenomena arising in the disorder.

To a subject laden with endless theory, the author brings convincing physiological evidence of a hypothalamic disturbance. He has given special emphasis to the hyper-ventilation index, a test which establishes the improvement in breath-holding after hyper-ventilation in normal persons.

PRESENTS

Strong physiological evidence in favor of the theory of cortico-hypothalamic imbalance

STUDIES

Mental status—Subjective dyspnea—Giddiness—Palpitation—Pain—Fatigue (mental and physical)—Sweating—Hyperthemia

272 pages $3.00

THE WILLIAMS & WILKINS COMPANY

Baltimore 2, Maryland

(In writing to advertisers, please mention the Journal—it helps.)

FOR CLASSROOM STUDY OR REFERENCE READING

SMITH and GAULT

Essentials of Pathology

Smith of Cornell Medical and Gault of Temple here present a new (third edition) textbook, which is ideally suited to the beginner in pathology because it is both "practical and restricted to absolute essentials." The Journal of the American Medical Association calls it —"an excellent textbook in that it enables students quickly to develop a firm foundation in clinically applicable pathology." New material has been added—case histories condensed and repetitious material reduced—61 new figures (four in color) appear. The ideal book in the subject for the new student and for those who wish to refresh their memories.

740 Illustrations, 764 Pages, $12.00

DYKE (Editor)

Recent Advances in Clinical Pathology

Four general sections (Biochemistry, Bacteriology, Hematology and Cytology, Histology) written by 40 of the world's well known clinical pathologists, and presented as a symposium—this book evaluates the whole field. Sheds important new light on practical problems of today. A new book.

34 Plates, 468 Pages, $5.50

HADFIELD and GARROD

Pathology

Presented here . . . new nomenclature, late researches in cancer, inflammation, site of antibody formation, silicosis, endocrines. There is recent work on hepatic diseases and dietary deficiencies. Many new illustrations. A new work in the Recent Advances Series.

60 Illustrations, 362 Pages, $6.00

HAWK, OSER and SUMMERSON

Practical Physiological Chemistry

Here's what the critics say about the new twelfth edition of this long-standard work . . . "remains the standard classic of its field" (International College of Surgeons) . . . "the coverage of subjects is broader than ever" (Science) . . . "This edition should enjoy the same popularity as earlier ones" (Annals of Internal Medicine) . . . "recommended to all who deal with biochemical procedures, their interpretation and significance" (Virginia Medical Monthly). To quote more is to gild the lily. But judge for yourself.

1323 Pages, 329 Illustrations, $10.00

THE BLAKISTON COMPANY

1012 Walnut Street, Philadelphia 5, Pa.



Please send the items checked: ☐ my check is enclosed ☐ charge my account
It is understood that you will credit my account in full for any books that I wish to return within 10 days.

☐ Smith and Gault—Essentials of Pathology ☐ Dyke—Recent Advances in Clinical Pathology
☐ Hadfield and Garrod—Pathology ☐ Hawk, Oser and Summerson—Practical Physiological Chemistry

NAME..... ADDRESS.....

CITY..... ZONE..... STATE.....

AJCP 4-49

(In writing to advertisers, please mention the Journal—it helps.)

PART-TIME JOBS WANTED!

Are you utilizing the services of trained medical technologists available in your community for part-time work?

Statistics on file in the Registry office show that only a small number of registered medical technologists now retired from active professional life, are working part-time in their field. By using these trained laboratory workers for vacation and sick relief, for night call, and for week-end duty, you may be able to increase the laboratory service you can offer to your community.

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
American Society of Clinical Pathologists
Muncie, Indiana

★

Experienced clinical laboratory technician: $3339; 40 hour week; day shift; 594 bed hospital; maintenance, $39 per month.

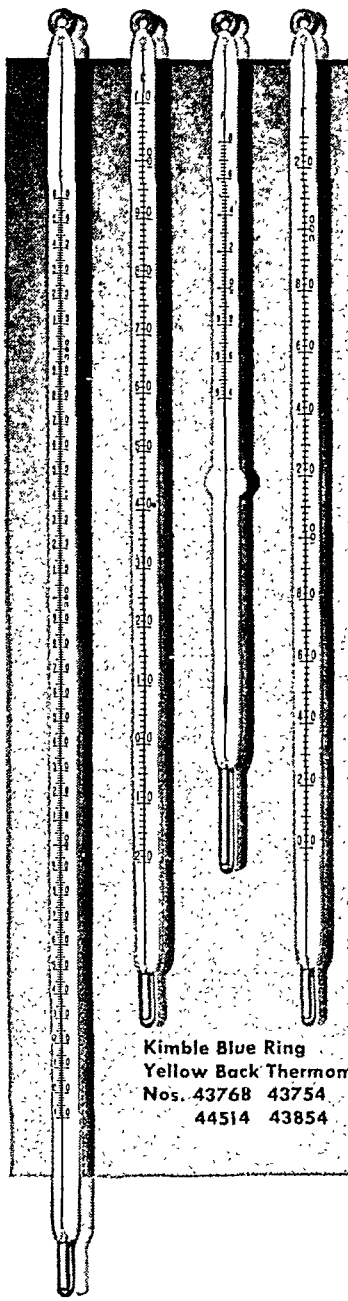
★

Write to
JOHN T. SMILEY, M.D.
SAN JOAQUIN GENERAL HOSPITAL
P. O. Box 1890
Stockton, California

Research Opportunity

A well trained research worker is required to conduct experiments in the biological aspects of 25 mev X-rays and electrons. A Betatron at the University of Saskatchewan is available. Applicants should be capable of independent research and, therefore, must have had previous experience in radio-biological research. The appointment is for a minimum of two years. Salary by arrangement. Further information may be obtained from: Dr. T. A. Watson, Director, Cancer Clinic, City Hospital, Saskatoon, Saskatchewan, Canada.

(In writing to advertisers, please mention the Journal—it helps.)



Kimble Blue Ring
Yellow Back Thermometers,
Nos. 43768 43754
44514 43854

Retested . . .
Accurate . . .
Readable . . .

Kimble Blue Ring Yellow Backs

are here!

KIMBLE GLASS . . . foremost maker of precision laboratory glassware . . . proudly presents this outstanding new line of retested, accurate, easily read thermometers. Every item in this new line is *retested* to insure its accuracy.

Precise! There is a Kimble Blue Ring Yellow Back Thermometer for each scientific specification—ASTM and other special-purpose thermometers—National Bureau of Standards thermometer specifications . . . can be N.B.S. Certified if required.

High visibility! The yellow back behind the column and the *new style horizontal* numbering insure speediest and most accurate reading . . . save your time and minimize possibility of error.

Look for the blue ring on top that instantly identifies genuine Kimble Yellow Backs.

If your laboratory supply dealer does not yet have Kimble Blue Ring Yellow Back Thermometers, write us for the names of the nearest dealers who have them.

KIMBLE GLASS TOLEDO 1, OHIO

Division of Owens-Illinois Glass Company

© 1949—O. I. G. Co.

LABORATORY TECHNIQUE

In Biology and Medicine

SECOND EDITION

By E. V. Cowdry

Professor of Anatomy, Washington University

An excellent laboratory instrument for the technician

- Expanded to include related physical methods and micro-chemical technique.
- New methods and improvements in standard techniques added.
- A laboratory tool that broadens the utility of every other piece of laboratory equipment in its scope.
- It is "definite information on specific matters," as the author states.
- And the definite information is really accessible—all alphabetically arranged under names of structures, and strains, and elements, and names of procedures. You can hardly miss.
- Thoroughly documented with the references where you want them—as part of the text you are reading at the moment.
- In short, as the *American Journal of Medical Technology* puts it, the worker "will find it useful at the work table instead of on the book shelf."

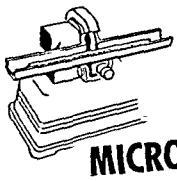
LABORATORY TECHNIQUE

Cloth 6 x 9 **224 pages** **self-indexing** **$4.00**

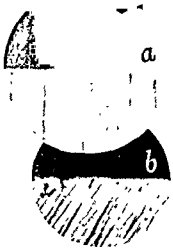
THE WILLIAMS & WILKINS COMPANY

Mt. Royal & Guilford Aves.

Baltimore 2, Maryland



the Berkeley
MICROTOME KNIFE SHARPENER
assures an
INCOMPARABLE EDGE
3 times faster...



COMPARE THE EDGES. Photomicrograph *(a)* shows a microtome knife edge sharpened by the Berkeley sharpener. Note its even smoothness, assurance of better sections. Photomicrograph *(b)* shows a blade laboriously hand sharpened, honed and stropped, but note the nicks and serrations still remaining.

COMPARE THE TIME. In tests, wherever made, and in practice in hundreds of laboratories, the Berkeley Microtome Knife Sharpener gives perfect edges in one-third the time required for hand honing and stropping.

Let us send you Bulletin MKS-14 giving full information about this indispensable laboratory aid.

Berkeley Scientific Company
SIXTH AND NEVIN AVE • RICHMOND, CALIFORNIA

SHILLABER'S
Non-Drying
IMMERSION OIL
for Microscopy

OFFERS THESE ADVANTAGES OVER CEDAR OIL..

- *Better Results
- *Safer
- *Time Saving
- *Neutral
- *Low Fluorescence
- *2 Viscosity Ranges
- *Controlled Optical Properties



PRICES (Grade A or Grade B)

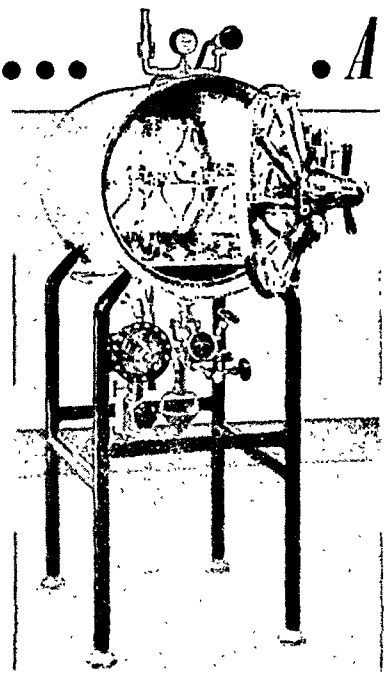
COMBINATION PACKET	
1 Ounce A with 1 Ounce B	$1.00
1 Ounce Bottle Each	.50
4 Ounce Stock Bottle	1.50
16 Ounce Stock Bottle	4.00

ONE OUNCE BOTTLES HAVE APPLICATOR RODS

Order Through Your Laboratory Supply Dealer Or Direct From

R. P. Cargille 118 Liberty Street New York 6, N. Y.

Castle ... *AUTOCLAVES*



Designed for precision laboratory work with complete safety and reliability.

Three functions: Air and Condensation Discharge valved to permit operation at temperature of pure steam; flowing steam; or steam and air for coagulation of serum.

Condensation hood over upper half of chamber prevents dripping on contents.

"Forced Air Evacuation" insures 100% steam circulation for sterilization . . . completely eliminates air and air pockets, guarantees uniformity of temperature.

Available in Direct Steam, Electric and Gas heated models . . . in single and double wall construction.

Full opening safety door . . . opens within 1½" of full diameter of chamber. Cannot open accidentally under pressure.

Extra heavy construction . . . with rugged fittings that operate easily, give long-lived service.

For full details of Castle Autoclaves and other Castle Bacteriological Apparatus, write: Wilmot Castle Co., 1216 University Ave., Rochester 7, N. Y.

Castle **BACTERIOLOGICAL APPARATUS**

Advertisers in the Journal Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.

ALBIMI

BLOOD CULTURE MEDIA

Prepared in the

"McCARTNEY BOTTLE"

not only

IDEAL FOR BED-SIDE USE

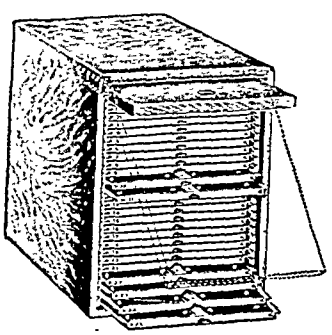
but also

SOLVES MAILING PROBLEM

Leaflet on request

ALBIMI LABORATORIES

460 SEVENTH AVENUE
BROOKLYN 15, N. Y.



Unit System Cabinet

No. 60-660
$36.00

EBERBACH
MICRO SLIDE CABINETS

The Unit System Cabinet provides *flat* storage for 500 3″ x 1″ slides. 25 aluminum trays are numbered, and each has 20 compartments. Hardwood cabinets with flat sides and disappearing door can be stacked as slide collection increases. They measure 8½ x 10 x 12″ deep. Write for Bulletin 150.

Eberbach & Son Company
LABORATORY APPARATUS & SUPPLIES
ESTABLISHED 1849
ANN ARBOR, MICH.

"VACSEAL"

MEANS DEPENDABILITY IN GUINEA PIG COMPLEMENT

Since 1939, Carworth Farms has been the largest producer of guinea pig complement in this country. We are now distributing this material under our own label, "Vacseal", at a more favorable price.

It is well known that the production of this biologic requires great care. Our long experience and large output enable us to offer a superior product to the laboratory worker. We invite you to try "Vacseal" Guinea Pig Complement, available in glass ampules with a special diluent for restoration to the following:

3 cc 7 cc 20 cc

Any recognized laboratory will receive a trial 3 cc package on request.

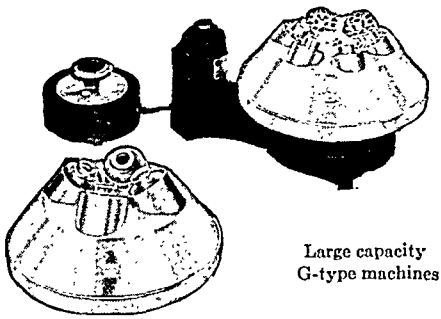
CARWORTH FARMS INC.
NEW CITY · ROCKLAND COUNTY · NEW YORK

(In writing to advertisers, please mention the Journal—it helps.)

SERVALL

ANGLE CENTRIFUGES

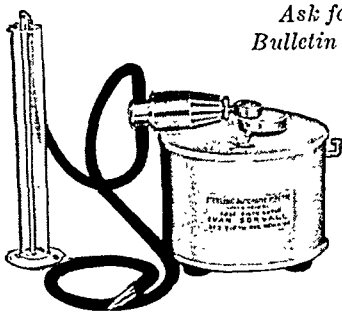
Ask for Bulletin J-41
with complete data on all models



Large capacity
G-type machines

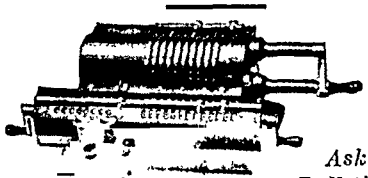
Type G/1, for 6 bottles of 250 cc each
18 tubes of 60 cc each
42 tubes of 15 cc each
78 tubes of 10 cc each
Type G/2, for 4 bottles of 500 cc each
Type G/3, for 10 tubes of 100 cc each

Ask for
Bulletin J-42



STERLING AUTOMATIC
AND
HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.
Easy to operate—Time Saving



Ask for
Bulletin J-43

ORIGINAL—ODHNER

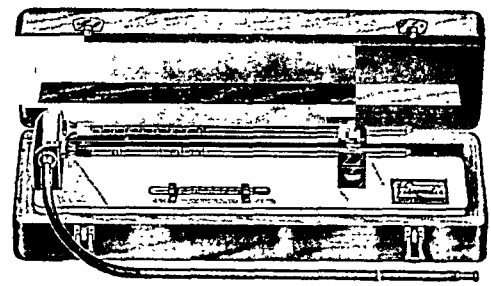
Portable Calculator for the Scientist
Many different models
Efficient - Sturdy - Low Priced

Manufacturers and Distributors:
IVAN SORVALL, Inc.
210 FIFTH AVE.-NEW YORK 10, N. Y.

NEW

HELLIGE
Viscometer

IMPROVED
HESS
BLOOD
VISCOSIMETER



The Hellige Viscometer is the first American-made instrument embodying improvements in the original Hess Blood Viscosimeter. Completely redesigned to provide greater simplicity and ease of operation, the Hellige Viscometer assures viscosity determinations of greater accuracy than were obtainable with the Hess model.

One of the new features is the viscometer tube, consisting of collecting, flow, and measuring portions sealed together in one complete unit, which can be handled as an ordinary pipette.

The accuracy of Hellige Viscometers is increased by addition of a special leveling device which eliminates the zero drift responsible for most of the erroneous determinations obtained with Hess Viscosimeters.

COMPLETE, $45.00

WRITE FOR CATALOG NO. 1200

HELLIGE
INCORPORATED
3718 NORTHERN BLVD.
LONG ISLAND CITY 1, N.Y.

Third Edition

A TEXTBOOK OF CLINICAL PATHOLOGY

Edited by the late FRANCIS P. PARKER, M.D.*

Because of the pressure of other duties, Dr. Roy Kracke, former chief editor, found it necessary to withdraw from the work for this third edition. The revision, however, in the capable hands of Dr. Parker, is complete—in fact, the book has been almost entirely rewritten. Changes involve the bringing up to date of material from former editions and the additions of new procedures developed in the last six years. Rearrangement of sequence in some sections has improved the continuity of the text.

*Dr. Parker's untimely death occurred just after he completed the reading of the page proof.

Emphasis on interpretation of laboratory findings
Contributors all experienced workers
Wide range of topics within relatively small compass

What the Reviewers said about the second edition

• An unusually fine textbook . . . that covers comprehensively, accurately and lucidly every aspect wherein the laboratory may aid in clinical diagnosis.—THE NEW ENGLAND JOURNAL OF MEDICINE

• Only the well-known standard procedures have been selected and they are described with accuracy and simplicity. The figures and tables have been assembled with a view to both beauty and clarity.—THE INTERNE

• It can be cordially recommended to medical students, physicians and laboratory workers as an accurate, useful and thorough consideration of clinical pathology.—AMERICAN JOURNAL OF TROPICAL MEDICINE

• The pleasing style, the attractiveness of the volume and the complete consideration of clinical pathology lead this reviewer to recommend this text for the teaching of medical students and as a reference for the medical technologist and the physician who does his own laboratory work.—SOUTHERN MEDICAL JOURNAL

1043 pp. *229 illus., 46 plates* *$9.00*

THE WILLIAMS & WILKINS CO

BALTIMORE 2, MARYLAND

(In writing to advertisers, please mention the Journal—it helps.)

AT HOME OR AWAY SPOT TESTS SIMPLIFY URINALYSIS

No Test Tubes • No Measuring • No Boiling

Diabetics welcome "Spot Tests", (ready to use dry reagents), because of the ease and simplicity in using. No test tubes, no boiling, no measuring; just a little powder, a little urine—color reaction occurs at once if sugar or acetone is present.

Galatest . . . Acetone Test (DENCO)

FOR DETECTION OF SUGAR IN THE URINE — FOR DETECTION OF ACETONE IN THE URINE

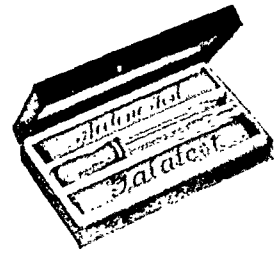
SAME SIMPLE TECHNIQUE FOR BOTH

1. A LITTLE POWDER  2. A LITTLE URINE

COLOR REACTION IMMEDIATELY

Accepted for advertising in the Journal of the A.M.A.

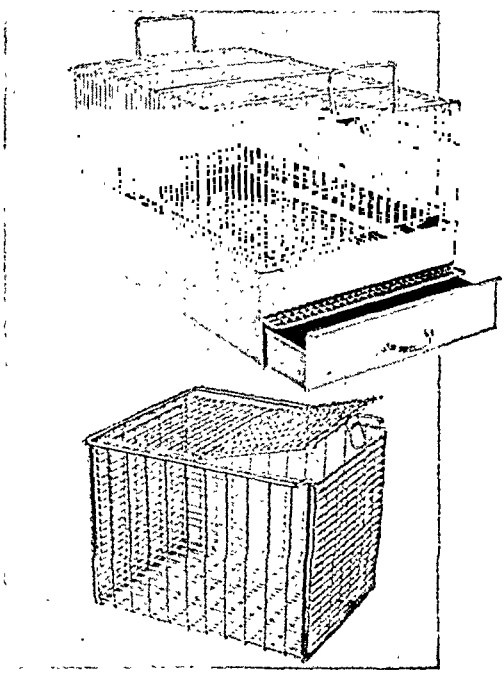
WRITE FOR DESCRIPTIVE LITERATURE



A carrying case containing one vial of Acetone Test (Denco), one vial of Galatest, medicine dropper and Galatest color chart is now available at all prescription pharmacies and surgical supply houses. This is very convenient for the medical bag or for the diabetic patient.

Acetone Test (DENCO) *Galatest* THE DENVER CHEMICAL MANUFACTURING COMPANY, INC. 163 VARICK ST., NEW YORK 13, N. Y.

FOR THE MOST DISCRIMINATING TECHNICIAN



Laboratory Animal Cages by *Bussey*



Bussey cages are the ultimate in cleanliness and ease of servicing. Several specific-purpose cages, one all-purpose cage...for guinea pigs, hamsters, rat colonies, small rabbits and cats. Racks designed for each type of cage. Cages are suspended, with dropping pans as separate units. Finest electric welded construction. Finish specially resistant to strong cleansing and disinfectant solutions. Write for literature and prices. Free consultation on special equipment problems. Write today.

Prompt Delivery

BUSSEY PRODUCTS CO.

6000-55 W. 51st St. • Chicago 38, Ill.

(In writing to advertisers, please mention the Journal—it helps.)

For the first time in one volume—complete and authentic information on

BLOOD DERIVATIVES and SUBSTITUTES

Including a Discussion of Shock

By CHARLES STANLEY WHITE, M.D.

Chief of Surgery, Doctors Hospital, Washington, D. C.

and

JACOB JOSEPH WEINSTEIN, M.D.

Associate in Surgery, George Washington University School of Medicine

No other one book in the literature has assembled such a wealth of usable information on this subject of rapidly increasing importance.

The chemistry and physiology of plasma are discussed clearly and in full detail, as is the role of the function of plasma protein. One section is devoted to laboratory procedures and the experimental and clinical study of blood and plasma.

The preparation, storage and use of plasma are given full coverage with a complete discussion of the clinical use of dry plasma. Routes of administration, including the intraosseous, are admirably described and well illustrated.

Plasma fractionation and fractions are treated in a special chapter.

The discussion of shock is complete and covers physiology, chemistry, pathology and clinical manifestations, with a particularly full and valuable section on treatment.

Nine blood substitutes are described in detail, with methods of administration.

530 pages *196 figures* *$7.50*

SANS TACHE

THE WILLIAMS & WILKINS COMPANY

Mt. Royal & Guilford Aves. Baltimore 2, Md.

BBL

TRYPTICASE SOY AGAR

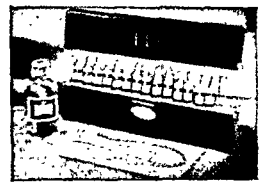
A general purpose medium, especially useful for determination of hemolysis and for the isolation of fastidious organisms.

Literature and prices sent on request

Baltimore Biological Laboratory

1640 Gorsuch Avenue Baltimore-18, Md.

LAMOTTE COMPARATOR FOR BLOOD LOSS IN BODY FLUIDS



A Special La Motte Outfit has been developed according to the method suggested by Dr. Kyril B. Conger (Department of Surgery, University of Michigan, Ann Arbor, Michigan).

The special comparator illustrated above contains twelve color standards calibrated according to the colorimetric scale, representing known amounts of blood in the diluted fluid. Marked comparator tubes are provided for the unknown fluid.

In making the test a measured portion of the unknown fluid is placed in the comparator tube and a reading made directly against the color standards in the comparator. This determines the amount of blood loss in the measured sample, and by simple multiplication (involving the total amount of the fluid collected) the total blood loss is determined.

The unit comes complete with full instructions, ready to use.

If you do not have the LaMotte Blood Chemistry Handbook, a complimentary copy will be sent without obligation upon request.

LaMotte Chemical Products Co.
Dept. "D" Towson, Baltimore 4, Md.

New Abstract Service

EXCERPTA MEDICA

Fifteen journals publishing pertinent and reliable abstracts in English of every article in every available medical journal

Journals cover Anatomy, Anthropology, Embryology and Histology; Physiology, Biochemistry and [illegible] and Medical [illegible] Pathological [illegible]edicine; Pediatrics; Neurology and Psychiatry; Surgery; Obstetrics and Gynecology; Oto-, Rhino-, Laryngology; Ophthalmology; Dermatology and Venereology; Radiology; and Tuberculosis.

Each journal is issued monthly, one or two volumes a year.

The Williams & Wilkins Company sole distributor for the United States, Canada and Central America.

Send for the free booklet giving complete information and descriptions of each journal

The Williams & Wilkins Company
Mt. Royal and Guilford Aves.
Baltimore 2, Md.

REICHERT MICROTOMES

It gives us great pleasure to announce that REICHERT MICROTOMES of Vienna are again available.

We are now in a position to accept orders for the following internationally recognized instruments:

REICHERT LARGE SLIDING TYPE MICROTOME "OmE"

REICHERT FREEZING TYPE MICROTOME "OmP"

Write now for full information to:

LABORATORY EQUIPMENT COMPANY

118 Eleventh Street San Francisco 3, California

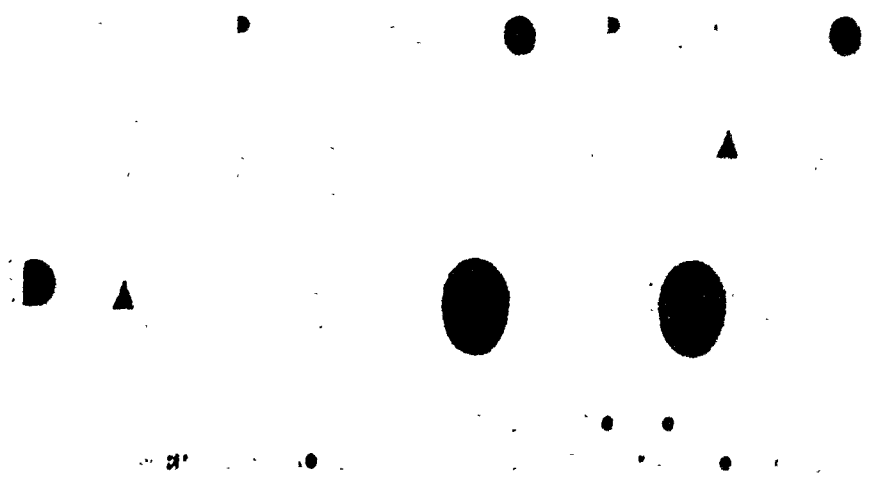
(In writing to advertisers, please mention the Journal—it helps.)

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia..O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DYRENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Medical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Benbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Canada.....S. M. ASSELSTINE, Medical Arts Bldg., Windsor, Ontario
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

VOLUME 19 MARCH 1949 NUMBER 3



S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R. BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology.*

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, 6060 Park Avenue, Indianapolis 5, Ind. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: **The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.**

Agents: For Australia: Angus and Robertson, Limited, 89–95 Castlereagh Street, Sydney; for the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 MARCH 1949 NUMBER 3

CONTENTS

Actinomycosis. A Pathologic and Bacteriologic Study of Twenty-One Fatal Cases. L. A. WEED AND A. H. BAGGENSTOSS 201

A Simple Direct Method for the Electron Microscopy of Peripheral Blood Cells. J. W. REBUCK 217

The Value of Beryllium Determinations in the Diagnosis of Berylliosis. F. R. DUTRA, J. CHOLAK AND D. M. HUBBARD 229

Needle Aspiration in the Diagnosis of Lung Carcinoma. Report of Experience with Seventy-Five Aspirations. E. Y. GLEDHILL, J. B. SPRIGGS AND C. H. BINFORD 235

Case Report: Malignant Pheochromocytoma and Hypertension. J. CHAMOVITZ AND H. FANGER 243

Selected Abstracts 252

Book Reviews 256

Books Received 258

Technical Section

A Method of Determining the Distribution of Erythrocyte Fragility in Human Blood. E. E. ECKER, C. W. HIATT AND L. M. BARR 259

Titration of Physiologic and Immune Antibodies with Washed and Unwashed Erythrocytes. J. GUREVITCH, Z. POLISHUK AND D. HERMONI 265

Methods of Concentrating Acid-Fast Bacilli. H. J. CORPER AND C. R. NELSON 269

Standard Kline Test of Cerebrospinal Fluid for Syphilis. Employing Cardiolipin-Lecithin Antigen. B. S. KLINE AND H. SUESSENGUTH .. 274

A Special Microslide for Culture of Fungi. M. L. LITTMAN 278

Use of *Xenopus laevis* in Testing for Pregnancy. Analysis of 1100 Tests. M. DITTEBRANDT 284

The Divided Culture Plate. J. FELSEN 289

Dioxane Method of Dehydration of Tissues for Paraffin Embedding. T. F. WALKER 291

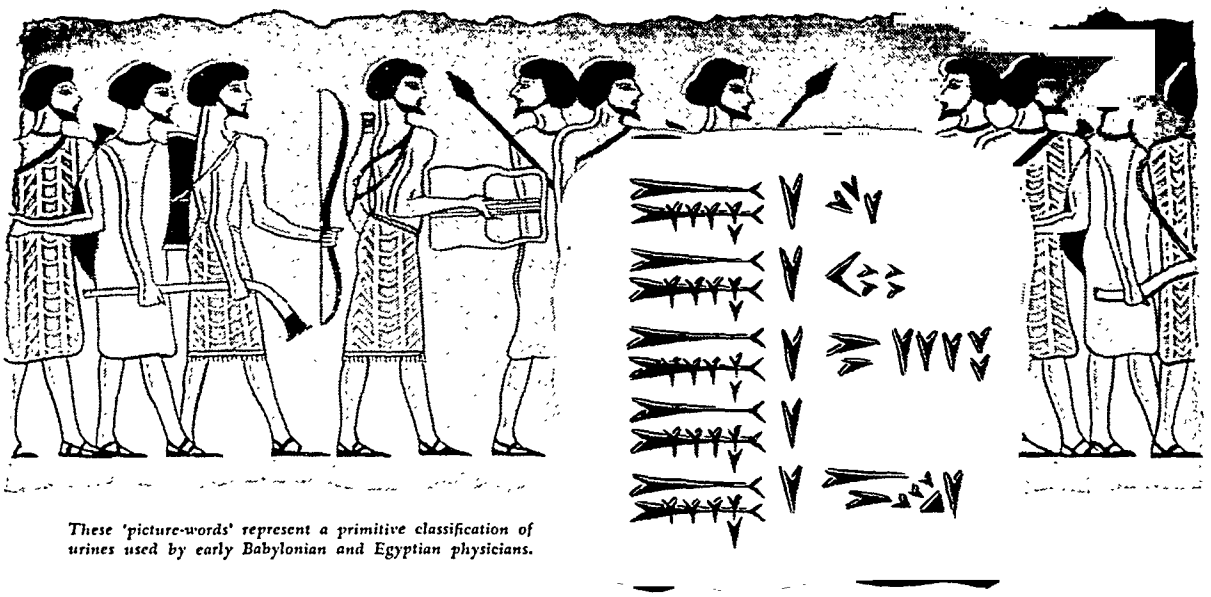
An Improved Simplified Technic for Collecting Multiple Blood Specimens. C. A. HUNTER AND F. VICTOR 293

Use of Silicone-Treated Test Tubes to Obtain Serum from Blood. R. D. TURNER, M. C. LAWLESS, E. CLEARY AND E. M. FRENCH 296

Preparation of Folin-Ciocalteu's Phenol Reagent. Using 30 Per cent Hydrogen Peroxide. D. O. ROSBASH 297

A Convenient Support for Sedimentation Tubes. E. M. ABRAHAMSON 298

News and Notices 300



These 'picture-words' represent a primitive classification of urines used by early Babylonian and Egyptian physicians.

centuries to perfect seconds to perform

When Sumerian and Babylonian physicians, *circa* 4000 B.C., noted the varying colors and constitutions of the "water of the phallus," they were probably not the first uroscopists in history. They were assuredly not the last, for fifty-odd centuries were to elapse before Fehling's first paper on the copper reduction test for urine-sugar appeared in 1848.

But centuries to perfect diagnostic procedures are condensed into seconds to perform the reliable *Clinitest*® method for urine-sugar levels. From start to finish, the test takes less than a minute. This tablet method is simplicity itself . . . readily learned by every diabetic patient. External heating is uniquely eliminated by the *Clinitest* procedure. Routine test interpretation is made easy.

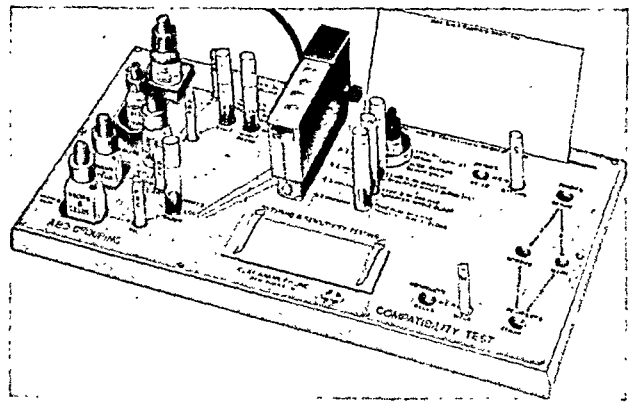
AMES

Clinitest

for urine-sugar analysis

AMES COMPANY, INC • ELKHART, INDIANA

BROWN Blood Grouping and Cross Matching BOARD



- **Minimizes the possibility of technical or clerical errors**
- **Embodies all the latest approved technics of blood grouping, Rh typing, Rh sensitivity testing and cross matching for transfusion.**
- **Simplifies and combines these technics in an orderly sequence.**
- **Facilitates the grouping and cross matching of blood for emergency or routine use.**
- **Can be used for routine Rh typing and sensitivity testing in individual maternity cases.**

The Brown Board[1] was developed in the Duke University Hospital Blood Bank to increase efficiency and reduce errors in blood typing and cross matching. It consists of an etched and stamped metal plate on a hardwood base, with an Rh typing box, anti-serum bottle adapters, reagent bottles, test tubes, dropping pipettes and concavity slides for Rh typing.

Tubes and reagent bottles have in-the-glass labels in different colors, matching the labelling and coloring of their places in the Board. Each reagent bottle and cross matching tube is of a different shape or size, so that it fits only in its proper place. Antiserum bottle adapters hold any commercial anti-serum bottle; they are permanently marked, fit only in their own place in the Board and are colored to conform to the coloring recommended for the sera by the National Institute of Health. Reagent bottles and adapters are designed to permit one-hand removal and replacement of the dropping pipettes.

The Rh typing box[2] is lighted, tiltable and designed to maintain a temperature of 37° to 42° C in the slide concavity where the blood sample is placed.

The Board has a slot to hold the Donor Card and Transfusion Request Form directly in front of the technician while tests are being made. A blotting paper holder is also provided, conveniently near the technician.

Refs: 1. "A Note on Blood Grouping and Cross Matching with Special Reference to a Convenient Cross Matching Board." Brown, I. W., Jr., M.D. In press.

2. "The Demonstration of Anti-Rh Agglutinins—An Accurate and Rapid Slide Test," Louis K. Diamond, M.D., Boston, Mass., and Neva M. Abelson, M.D., Philadelphia, Pa., Journal of Laboratory and Clinical Medicine, vol. 30, No. 3, March 1945.

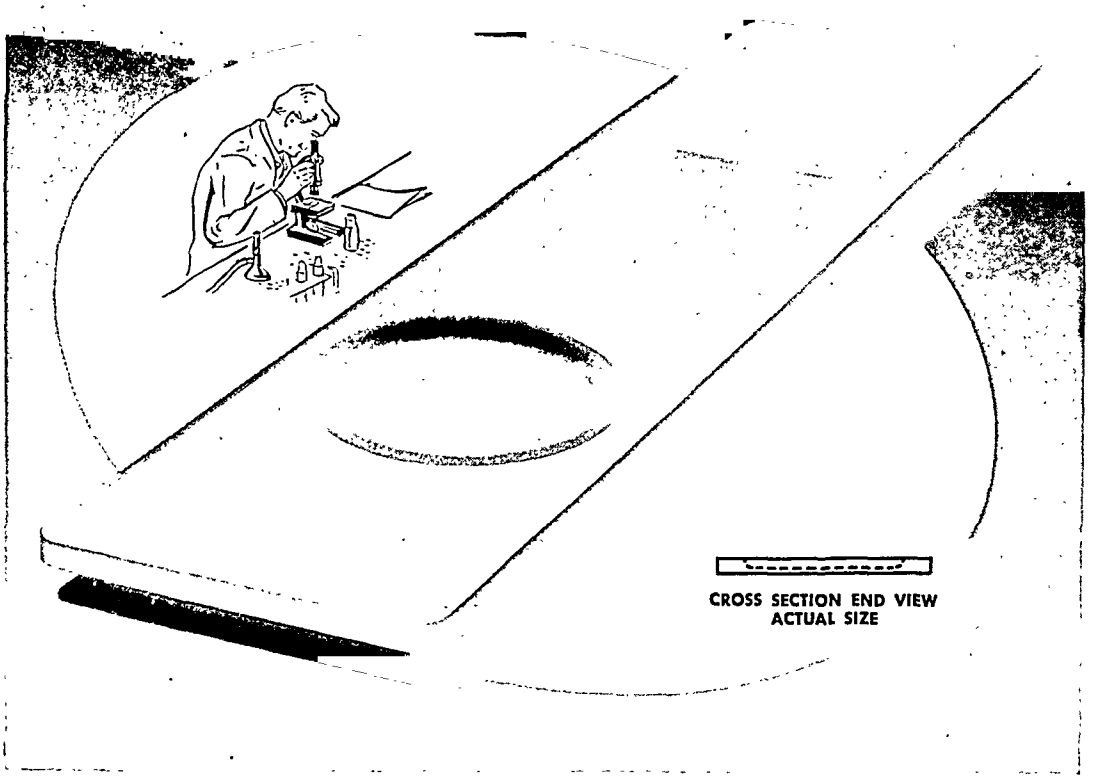
A-2800 Brown Blood Board, complete with Rh typing box, anti-serum bottle adapters, reagent bottles, test tubes, dropping pipettes and four-concavity slides. **Each $90.00**

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

(In writing to advertisers, please mention the Journal—it helps.)

A New Type MICRO SLIDE...

. . . for the primary isolation of pathogenic fungi from clinical specimens



The circular, flat-bottomed well of this slide is so shallow that all planes of the chamber (formed with a cover glass) can be brought into focus, yet it is deep enough to grow fungi satisfactorily.

See Dr. M. L. Littman article on the new slide in this issue!

Headquarters for Laboratory Supplies

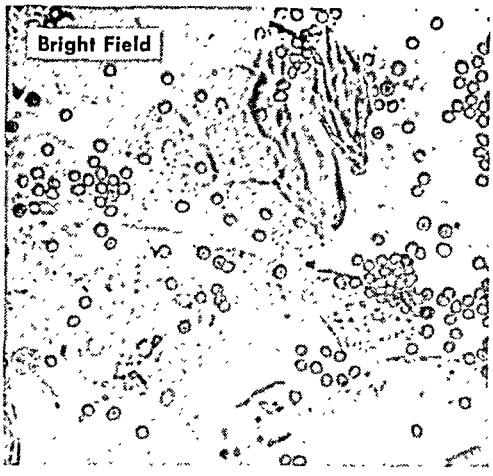
FISHER SCIENTIFIC CO.
717 Forbes St., Pittsburgh (19), Pa.
2109 Locust St., St. Louis (3), Mo.

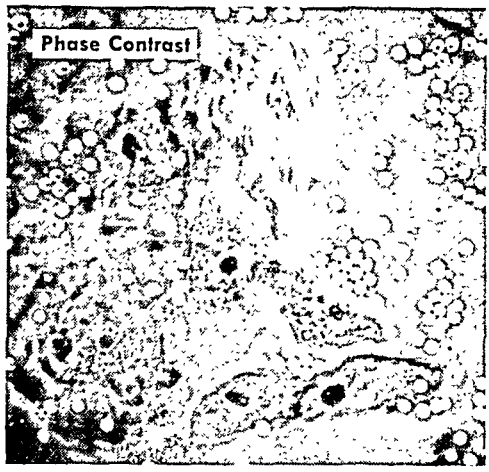
EIMER AND AMEND
Greenwich and Morton Streets
New York (14), New York

In Canada: Fisher Scientific Co., Ltd., 904 St. James Street, Montreal, Quebec

(In writing to advertisers, please mention the Journal—it helps.)

• *Sediment from centrifuged specimen of urine. Phase contrast more clearly differentiates between types of epithelium and casts, and shows presence of pus cells and bacteria.*





Here's TOP IMAGE CONTRAST

with ANY thin transparent specimen

TOP image contrast with any thin *transparent specimen* is obtained by means of the ¼ wave length annulus. This is conclusively verified by findings of recognized authorities, *plus* the theory of diffraction gratings, *plus* actual interferometer tests. Maintaining this critically precise coating is possible only by B&L high mechanical and optical precision.

Best results are also obtained by positive *contrast image* (denser substances are seen as darker tones), and *balanced intensity of direct and diffracted light beams.* This is the result of B&L accurately controlled coating processes. Of course, you want *speed, simplicity, and ease of operation.* You have it with B&L simplified accessories.

There are just four objectives, a



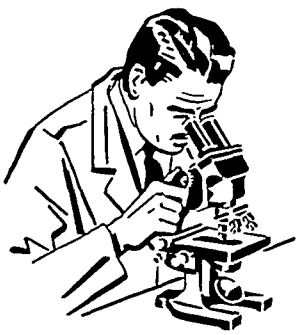
condenser assembly, auxiliary telescope and filter. It takes only a few minutes to put into operation, and is adaptable to most standard microscopes equipped with a substage rack and pinion.

Write for demonstration, and literature to Bausch & Lomb Optical Co., 652-C St. Paul St., Rochester 2, N. Y.

BAUSCH & LOMB *Simplified* PHASE CONTRAST ACCESSORIES

(In writing to advertisers, please mention the Journal—it helps.)

another **ALOE** exclusive

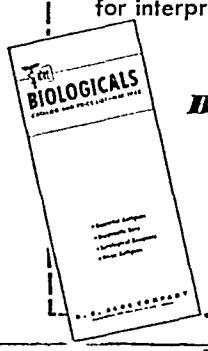


Experience and intensive research bring you Markham Diagnostic Biologicals

The Markham Diagnostic Biologicals . . . virus antigens and anti-sera . . . bacterial antigens and anti-sera . . . serological accessories including preserved sheep cells are now available for more exact confirmatory diagnosis. Each product is prepared to the highest standards and is fully guaranteed as to specificity and titre.

Standard Technique

With virus antigens, the principles of the well known complement-fixation technique are employed. The rapid slide or the macroscopic test tube method is used with bacterial antigens and anti-sera. Each vial is furnished with complete directions including suggestions for interpreting results.



FREE BIOLOGICAL CATALOG

Write for your free copy of complete catalog on Markham Diagnostic Biologicals No. 201.

Virus Antigens Anti-Viral Sera

Eastern Equine Encephalomyelitis
Western Equine Encephalomyelitis
Influenza "A"
Influenza "B"
Lymphocytic Choriomeningitis
Lymphogranuloma Venereum
Mumps, Epidemic Parotitis
Psittacosis-Human Pneumonitis Group
St. Louis Encephalitis
Rabies

Serological Accessories

Amboceptor, Anti-sheep Hemolysin
Guinea Pig Complement
Washed Standardized Sheep Cells

Bacterial Antigens

Brucella Abortus
Brucella Melitensis
Paratyphoid A
Paratyphoid B
Tularense
Typhoid, H and O
Preserved Sheep Cells
Proteus OX19

Anti-Bacterial Sera

Brucella Abortus
Brucella Melitensis
Paratyphoid A
Paratyphoid B
Tularense
Typhoid, H and O
Klebsiella pneumonia, Friedlander bacillus

a. s. aloe company *Serving Science Since 1860*

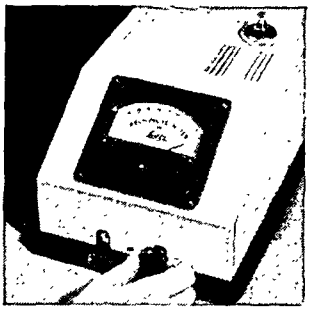
1831 Olive St • St Louis, Mo.

aloe

(In writing to advertisers, please mention the Journal—it helps.)

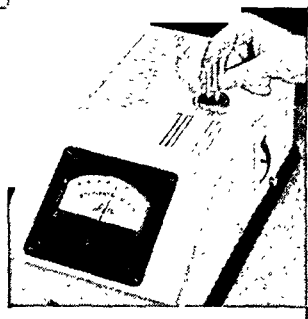
Clinical Chemistry Determinations

become simple as . . .



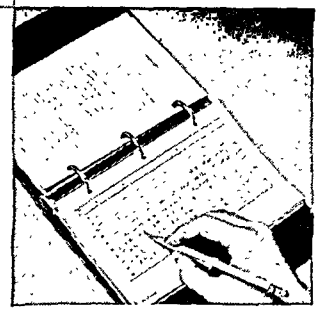
a

Set the needle to 100 after placing an absorption cell containing distilled water in the Photrometer.



b

Note the reading after replacing the cell of distilled water with a cell containing the unknown.



c

Refer to the table in the Handbook which indicates the concentration of the unknown for the particular reading.

with the

ROUY **PHOTROMETER**

by Leitz

the **PHOTROMETER** is individually calibrated for 38 of the most common clinical tests and can be readily calibrated for additional tests.

the **PHOTROMETER** is *pre-calibrated,* which makes it unnecessary to prepare or to keep a supply of standard solutions on hand. Accurately prepared reagents are quickly available through the Leitz Solution Supply Service.

the **PHOTROMETER** is furnished with its individual Handbook. It contains calibration tables that eliminate any need for calculation and outlines accepted clinical methods.

the **PHOTROMETER** is built to the exacting Leitz standards of craftsmanship and precision.

For information, write

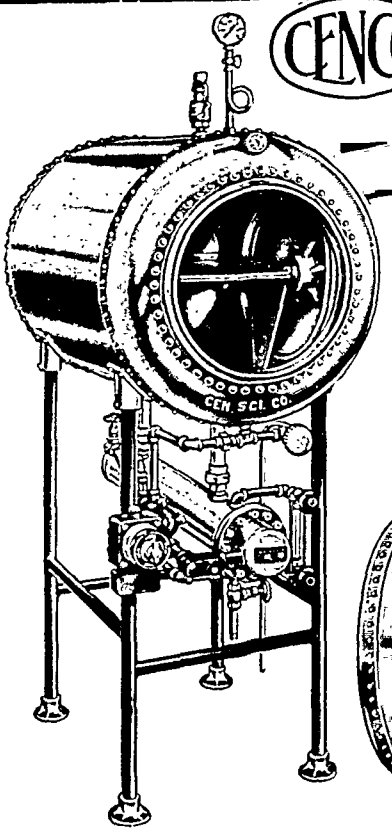
E. LEITZ, Inc., 304 HUDSON STREET, NEW YORK 13, N.Y.

LEITZ MICROSCOPES • BINOCULARS • SCIENTIFIC INSTRUMENTS
LEICA CAMERAS AND ACCESSORIES

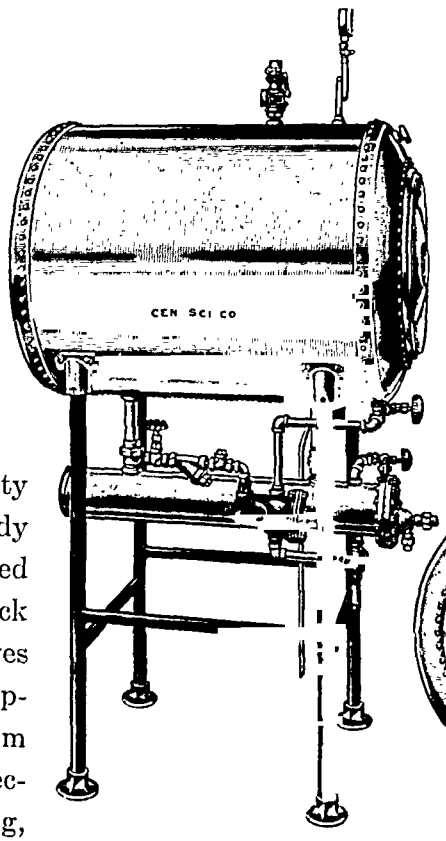
(In writing to advertisers, please mention the Journal—it helps.)

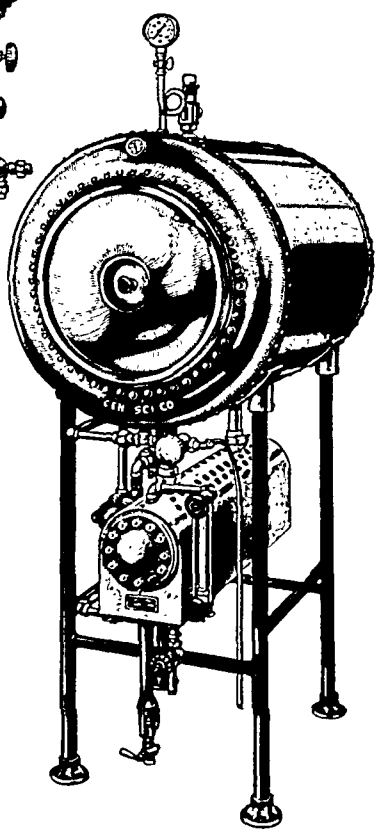
CENCO

HORIZONTAL AUTOCLAVES



COMBINE SPEED - SAFETY - CONVENIENCE. New design incorporates latest features for dependable laboratory operation. Cenco autoclaves permit rapid steam sterilization of culture media, glassware, apparatus, etc. in bacteriological, biological or clinical laboratories.





Meet standard safety specifications; body made of heavy rolled copper with thick block tin coating inside; shelves of monel metal. Supplied with separate steam generators for gas, electric or steam heating, constant water level device, dial thermometer, pressure gage, and safety valve. Made in two sizes: 28 x 21″ inside with 12″ door and 34 x 26″ inside with 16″ door. Economically priced.

WRITE FOR DESCRIPTIVE CIRCULAR 1187.

CENTRAL SCIENTIFIC COMPANY

Scientific (ENCO) Apparatus

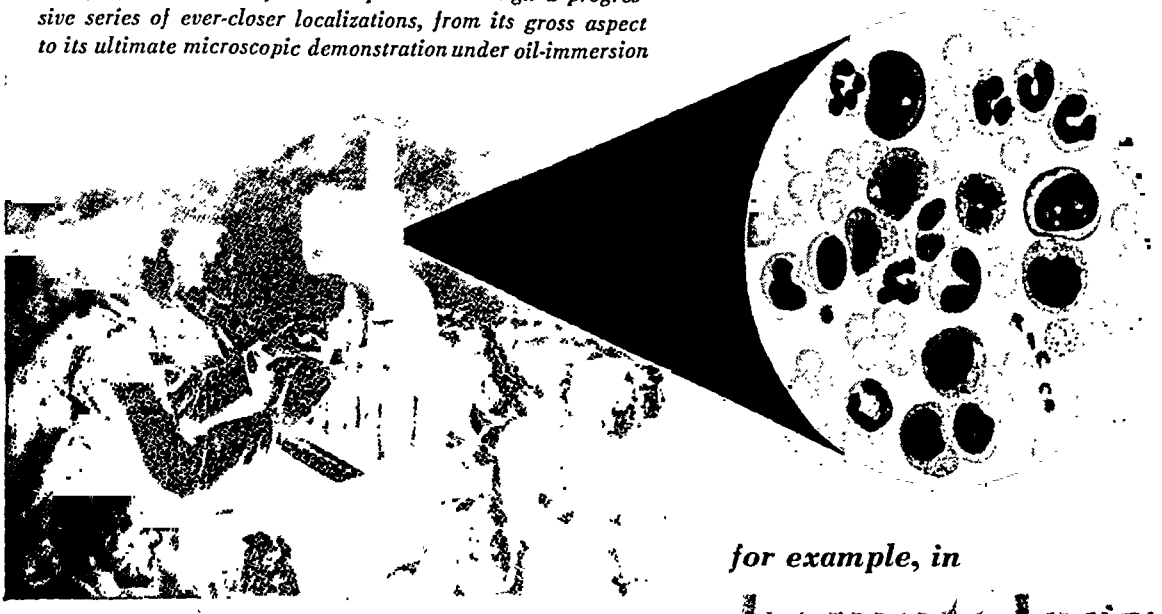
1700 IRVING PARK ROAD CHICAGO 13

NEW YORK BOSTON SAN FRANCISCO NEWARK LOS ANGELES TORONTO MONTREAL

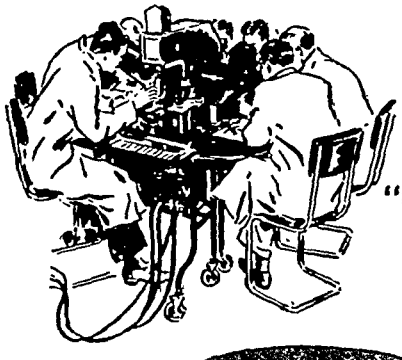
(In writing to advertisers, please mention the Journal—it helps.)

mass demonstration in the auditorium

The projectionist can follow a specimen through a progressive series of ever-closer localizations, from its gross aspect to its ultimate microscopic demonstration under oil-immersion



The auxiliary Scopicon dark-chamber "round-table" affords superb facility for small-group study. Here the projected image is cast upon a platen within a light-tight chamber, with separate light-excluding viewing hoods for each observer. Any number up to ten may share in conference, which can be conducted in a normally lighted room. A pointer at each port permits any observer to indicate areas of interest to all the others for discussion.



. . . and for small-group "round-tables" in a normally lighted room

for example, in

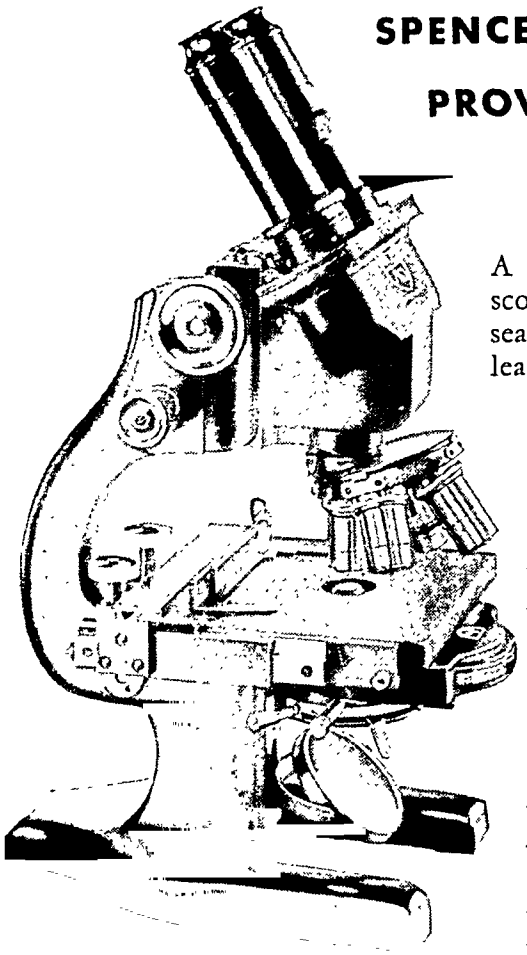
hematology

The 64,000 lumen intensity of the Scopicon high-pressure mercury lamp permits auditorium demonstration of blood smears even under oil-immersed microscope objectives. In projected images six feet or more across, the minute granular details of white blood cells can be seen, due to the pinpoint character of the 1 mm. square (approx.) focal spot employed. The Scopicon light is steady, flickerless . . . its brilliant white color exhibiting biological stains to the greatest advantage. May we send you the brochure describing this versatile instrument?

the Scopicon

SCOPICON, Inc.
215 East 149th St., New York 51, N. Y.

microprojection photomicrography



SPENCER PHASE MICROSCOPY PROVES MORE VERSATILE

A comprehensive study of 72 phase microscope applications, based on extensive research in cooperation with some of the nation's leading scientists, discloses—

IN 41 DIFFERENT APPLICATIONS Bright Contrast Objectives, in either low, medium, or high, give best results.

(AVAILABLE FROM AO ONLY)

IN 10 DIFFERENT APPLICATIONS Dark Contrast, Medium Objectives give best results.

(AVAILABLE FROM AO AND OTHERS)

IN 9 DIFFERENT APPLICATIONS B Minus Contrast Objectives, in either low, medium, or high, give best results.

(AVAILABLE FROM AO ONLY)

IN 2 DIFFERENT APPLICATIONS Dark Contrast, Low Objectives give best results.

(AVAILABLE FROM AO ONLY)

IN 10 DIFFERENT APPLICATIONS Bright or Dark Contrast give equally good results.

WRITE FOR FREE COPY OF THIS VALUABLE STUDY
(in easy-to-read chart form)

72 Specific Applications and recommended phase objectives with Phase Microscope bibliography revised to January, 1949. Write Dept. P16.

Here is up-to-the-minute evidence of the need for a wide variety of objectives to cover the many known applications for Phase Microscopy. The American Optical Company, *alone in the world today*, offers this variety from regular production. That is why there are many materials in which significant details can be seen only with Spencer Phase Microscopes. They are the logical choice for your laboratory.

American Optical Company, *Scientific Instrument Division*
BUFFALO 15, NEW YORK

For Leadership in Phase Microscopy the World Looks to AO **SPENCER**

C. I. No. 925

TOLUIDINE BLUE O

Certified for use in: Histology

..... GMS

Pharmaceutical Laboratories

NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A.

Found satisfactory by BIOLOGICAL STAIN COMMISSION for above-mentioned purposes. *This does not imply approval for any medicinal use.* Will users please report any unsatisfactory results to *H. J. Conn, Geneva, N. Y.*

Total Dye Content%

Certification No.
NU



For a rapid examination of experimental brain material, use *TOLUIDINE BLUE O.*

National

THE COMPLETE LINE OF
COMMISSION CERTIFIED BIOLOGICAL STAINS

NATIONAL ANILINE CHEMICALS PHARMACEUTICALS

Pharmaceutical Laboratories

NATIONAL ANILINE DIVISION

40 RECTOR STREET • • ALLIED CHEMICAL & DYE CORPORATION • • NEW YORK 6, N. Y., U.S.A

C&B

LABORATORY REAGENTS



New Catalog
FREE

If you have not received a copy of this new catalog please notify us at once.

Inorganic and
Organic Chemicals

•

Biological Stains
Solutions

•

Chemical Indicators
Test Papers

•

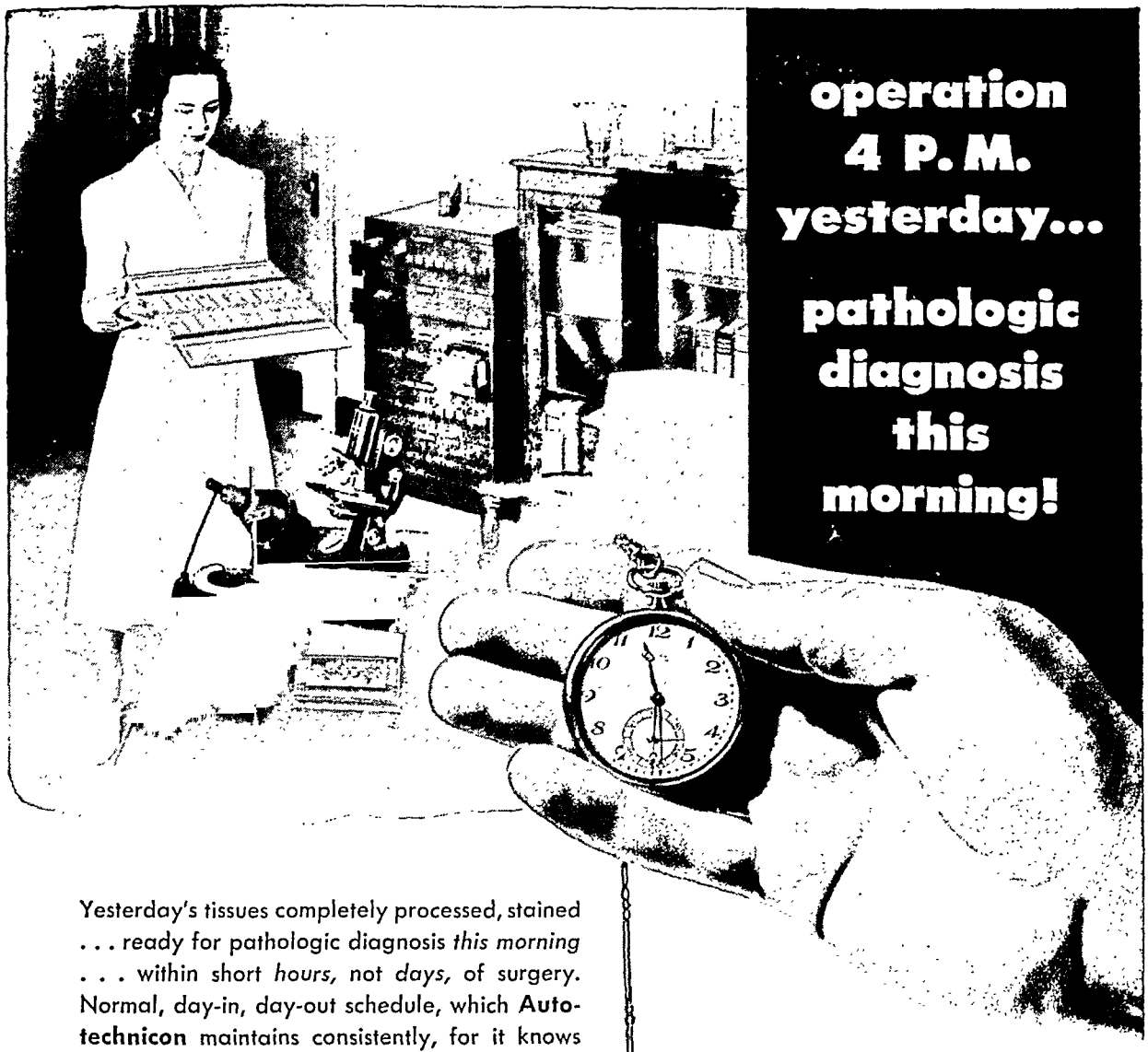
Distributed by
Physician and Laboratory Supply Houses

The COLEMAN & BELL Co., Inc.

Manufacturing Chemists : Norwood, Ohio, U. S. A.

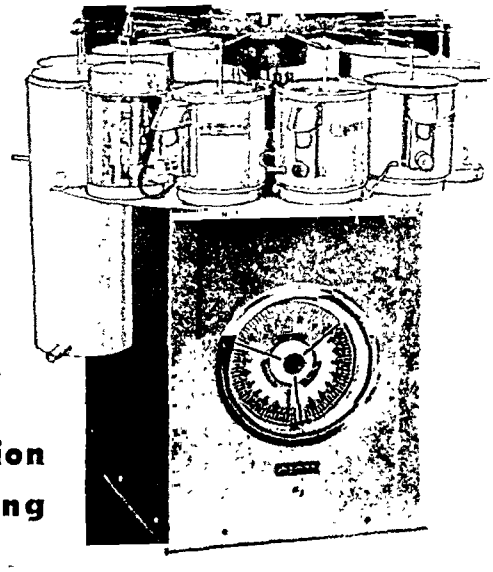
COLEMAN & BELL

(In writing to advertisers, please mention the Journal—it helps.)



operation 4 P.M. yesterday... pathologic diagnosis this morning!

Yesterday's tissues completely processed, stained . . . ready for pathologic diagnosis *this morning* . . . within short *hours,* not *days,* of surgery. Normal, day-in, day-out schedule, which **Autotechnicon** maintains consistently, for it knows no human fallibility, no human fatigue. Dispenses with tedious hand methods, with an overall gain in quality. The surgeon, as well as the pathologist, will find our brochure describing **Autotechnicon** of great interest. It is available on request.



Autotechnicon

Trade Mark Registered U. S. Patent Office

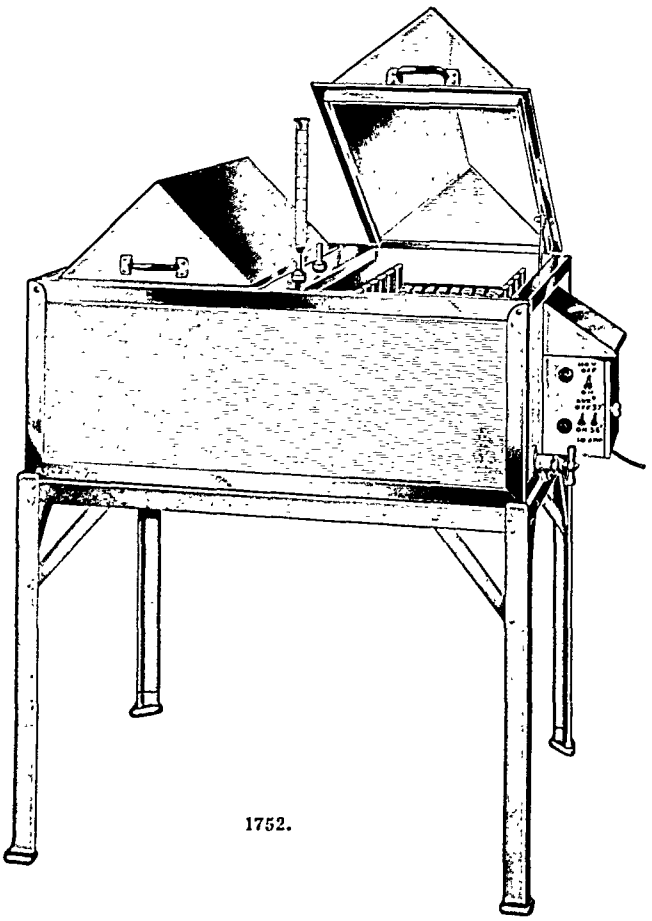
automatic fixation, dehydration washing, infiltration, staining

THE TECHNICON COMPANY
215 East 149th Street • New York 51, N. Y.

A.H.T. CO. SPECIFICATION

ELECTRIC SEROLOGICAL BATH

IN OUR STOCK FOR IMMEDIATE SHIPMENT



1752.

SEROLOGICAL BATH, Electric, A.H.T. Co. Specification. An improved design of the Wassermann Bath, usually referred to as the "U. S. Army Laboratory Model," originally described in *Bulletin 8, Surgeon General's Office, "The Prevalence of Syphilis in the Army,"* and retaining the advantage of a large volume of water.

Inside dimensions are 31¼ inches long x 24¼ inches wide x 12 inches deep, accommodating twenty No. 9492 Wassermann Racks or ten No. 1725 Kolmer Racks.

The bath is constructed throughout of heavy, tinned copper, insulated with air-cell asbestos 1-inch thick and covered with hard asbestos board finished with light gray enamel. Mounted on angle iron stand 29½ inches high, with cast iron feet.

The temperature is automatically controlled, at 37°C to ±0.15°C for incubation or at 56°C to ±0.25°C for inactivation, by means of a sensitive bimetallic thermo-regulator, the expanding member of which extends through half the length of the water bath.

The thermo-regulator is provided with heavy contact points, with the "make and break" mechanism enclosed outside the bath in a readily accessible metallic box. Arcing is prevented by means of a condenser. Adjustment for either 37°C or 56°C is accomplished by means of an indicating dial.

Because of the large volume of water with which this bath operates, the disposal of the water of condensation, which accumulates on the inside of the lids, becomes an important consideration. By means of new gable type lids, accumulated moisture is drained away from the contents of the tubes.

The heating units dissipate a total of 10 amperes at 115 volts when the auxiliary resistor is cut into the circuit for rapid heating by means of the switch.

1752. **Serological Bath, Electric, A. H. T. Co. Specification,** for operation at either 37°C or 56°C. Complete with twenty Wassermann Racks and Thermometer, range 25 to 70°C in 0.5°C, with red lines at 37° and 56° and with stem 180 mm long. With cord and plug. For operation on 115 volts, a.c. or d.c. 516.00

1753. **Ditto,** with thermometer but without racks 455.00

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

CABLE ADDRESS, "BALANCE," PHILADELPHIA

ACTINOMYCOSIS

A Pathologic and Bacteriologic Study of Twenty-One Fatal Cases*

LYLE A. WEED, M.D., and ARCHIE H. BAGGENSTOSS, M.D.

From the Section on Bacteriology and the Section on Pathologic Anatomy, Mayo Clinic, Rochester, Minnesota

Actinomycosis was one of the first diseases recognized as being of microbial origin. Bollinger is credited with first having recognized the organism as probably related to lumpy jaw in cattle. The granules were studied morphologically by Harz, who suggested the term "actinomyces" to describe the appearance of the filaments radiating from a central tangled mass. Soon thereafter, Israël recognized the organism in exudates from human material but was unwilling to say that it caused the disease process because other organisms were also present. However, Ponfick made such an interpretation in a human case and thereafter medical men began to associate lumpy jaw in cattle with actinomycosis in human beings as separate manifestations of one organism. These studies were all based on morphology and staining reactions of the granules, which are today recognized as being of definite but limited value in the accurate identification of an organism. In 1890 Bostroem claimed that he had cultured the organism and that it grew aerobically at room temperature. Furthermore he claimed to have isolated it many times from grains, grasses and soils, thus explaining the association of the organism with trauma from straws and from awns of grain. However, Wolff and Israël claimed that the organism they isolated would not grow at room temperature and required reduced oxygen tension.

Wright in 1905 reported that the organisms found in 13 cases of actinomycosis in man and cattle were all of one species, grew only at 37 C. and were essentially anaerobic in nature. Others have since amply confirmed Wright's work. Hagan and his colleagues have studied actinomycosis extensively and found that the lesions associated with the anaerobic, branching, filamentous organism occur as lumpy jaw in cattle, as mastitis in sows and as the various forms encountered in human beings. On the other hand, aerobic, branching filaments (probably Nocardia according to present-day terminology) have been clearly demonstrated many times as being etiologically related to certain human infections which have been called actinomycosis. Thus two organisms with somewhat similar morphologic features but distinctly different biologic properties have come to be associated with the condition known clinically as actinomycosis. This has been confusing to the clinician and laboratory worker alike. Experienced bacteriologists have long recognized the fundamental biologic differences in these two organisms, which can be demonstrated by cultural features, virulence, and in some cases

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, December 30, 1948.

staining reactions. It is desirable, therefore, to differentiate carefully the two groups of organisms and the disease processes with which they are respectively associated.

Whereas in the past it has been customary to consider both types of organisms as having the generic name Actinomyces, the sixth edition of *Bergey's Manual of Determinative Bacteriology* recognizes certain fundamental biologic differences in the strains and assigns them separate names according to the following scheme:

Family Actinomycetaceae
Genus I—Nocardia—obligate aerobic, sometimes acid-fast.
Genus II—Actinomyces—anaerobic or micro-aerophilic.

None of the organisms in genus II have to our knowledge been found to be acid-fast. The advantage to the clinician in following this terminology is emphasized later in the discussion.

The disease processes resulting from infection by these organisms differ epidemiologically and clinically. Bostroem claimed to have isolated the aerobic organism from many sources in nature, but Wolff and Israël claimed that the anaerobic form was found only in the animal body. These views are still recognized as valid interpretations. Many attempts have been made to isolate the anaerobic form from grasses and grains but without success. On the other hand, it has been found many times in association with various parts of the normal gastrointestinal tract.[5,12,25,26,27,31] Recently Kay found sulfur granules of the anaerobic type in 109 of 240 patients with pulmonary diseases of various types at Kennedy General Hospital, Memphis, Tennessee. Likewise the aerobic form is not commonly found in association with the normal animal body but is common in grasses, grains and soils. Therefore, since the actinomycosis caused by *Actinomyces bovis* (anaerobic form) is commonly associated with some lesion (dental decay or extraction, aspiration pneumonia, gangrenous appendix), it would appear logical that the organisms gain entrance to the tissues as part of the contaminating normal flora of the gastrointestinal tract of that particular person. Likewise, infection with the aerobic form would appear to be derived from some source outside the animal organism. For the same reasons one must exercise considerable judgment in evaluating the clinical importance of "sulfur granules" found in exudates from lesions associated with the respiratory or gastrointestinal tract.

In times past, the diagnosis of actinomycosis was made on either a histologic basis or the finding of sulfur granules in exudate from the lesion. There has been no rigid rule as to what constitutes such a granule and, therefore, the diagnosis of actinomycosis has varied considerably. With the development of selective chemotherapeutic and antibiotic agents it has become necessary to establish precisely the nature of the etiologic agent in any infectious process. Because the clinical pathologist is charged with this responsibility, we should like to present material emphasizing the importance of thorough bacteriologic investigation to supplement morphologic studies in cases of suspected actinomycosis.

Thirty-five cases in the files of the Section on Pathologic Anatomy were en-

countered in which the diagnosis of actinomycosis had been made. Twenty-one (Table 1) had typical gross and histologic patterns with organisms fulfilling the usual descriptions of *Actinomyces bovis*. Three had gross and histologic patterns usually associated with *Actinomyces bovis*, but the organisms encountered could not accurately be considered to be of this species. Nine had gross and histologic patterns resembling those of *Actinomyces bovis*, but no organisms could be demonstrated on repeated sections. Two had gross and histologic features of nocardiosis, one of which was proved by bacteriologic studies. These four groups are,

TABLE 1

ORGANS INVOLVED WITH *Actinomyces bovis* IN CASES OF ACTINOMYCOSIS

CASE	LUNGS	LIVER	KIDNEYS	OVARIES	CECUM	CENTRAL NERVOUS SYSTEM	VERTEBRAE	CAUSE OF DEATH*
1		+						Septicemia
2		+						Peritonitis
3		+						Septic hepatitis
4	+					+		Meningitis
5		+						Liver abscesses
6	+	+				+		Meningitis
7	+	+	+					Thrombosis of hepatic veins
8								Meningitis
9	+	+		+				Peritonitis
10		+						Pericarditis
11	+	+	+					Lung abscesses
12	+	+	+					Peritonitis
13						+		Meningitis
14	+	+	+					Pneumonia and liver abscesses
15	+	+	+					Liver abscesses
16	+					+		Meningitis
17	+							Brain abscesses
18	+	+		+				Thrombosis of hepatic veins
19	+				+		+	Lung abscesses
20	+							Lung abscesses
21				+				Brain abscesses

* Cause of death refers to the most significant or primary cause.
\+ Indicates involvement of organ.

therefore, referred to as (1) typical cases of actinomycosis, (2) equivocal or possible cases of actinomycosis, (3) cases apparently caused by some other etiologic agent but simulating actinomycosis, (4) cases of nocardiosis. For comparative purposes the pathologic features of one case from each of the four groups will be presented.

HISTOLOGIC FEATURES TYPICAL OF INFECTION WITH *Actinomyces bovis*

Case 1

The patient, a woman aged 35, had pneumonia in February with slow and incomplete recovery with associated weakness, nervousness and irritability. Tonsillectomy, per-

formed in November, was followed in two weeks by headache and inability to use the right arm, drooping of the left eyelid, followed by drowsiness, coma and death. At necropsy the following anatomic diagnoses were made: Multilocular actinomycotic abscess of the left ovary; multiple abscesses of brain (left frontal, temporal and occipital lobes); increased intracranial pressure (flattening of convolutions and narrowing of sulci); early cirrhosis of liver with arteriolosclerosis; focal interstitial suppurative nephritis of left kidney.

Histologic examination of the ovary revealed extensive destruction of tissue by multiple large and small intercommunicating abscesses (Fig. 1). The central collections of intact and degenerating polymorphonuclear cells often surrounded typical colonies of organisms (sulfur granules). Eosinophilic clubs were poorly developed but gram-positive branching filaments typical of *Actinomyces bovis* were revealed by use of the Brown-Gram stain. They were not acid-fast. Surrounding the central collections of polymorphonuclear cells there was a wall of granulation tissue consisting of fibroblasts, numerous lipophages and variable numbers of lymphocytes, plasma cells and polymorphonuclear leukocytes together with an occasional giant cell. Some of the abscesses were surrounded by a wall composed of denser connective tissue with collagen fibers.

Histologic examination of the brain revealed abscesses which were surrounded by a thin layer of loose vascular connective tissue containing a few fibroblasts with collagen fibers, many lymphocytes and plasma cells. In the surrounding brain there was a diminution in nerve cells, edema and an increase in microglia and astrocytes. Many of the latter were swollen with eccentric nuclei and deeply eosinophilic cytoplasm (so-called gemästete glia). No colonies of Actinomyces could be found.

Examination of the left kidney revealed acute focal suppurative interstitial nephritis (pyelonephritis).

EQUIVOCAL OR POSSIBLE CASE OF ACTINOMYCOSIS

Case 2

The patient, a man aged 59, after having had recurring pain in the right lower quadrant of the abdomen for six months, underwent operation for "ruptured appendix." An enterocutaneous fistula developed and persisted. Two months after operation empyema, loss of weight, fever and subdiaphragmatic abscess developed. The patient died nine months after onset with multiple lung abscesses. At necropsy the following anatomic diagnoses were made: Old (1943, elsewhere) appendectomy and later (elsewhere) drainage of right side of the chest for empyema; drainage of subdiaphragmatic abscess and drainage of abscesses of the right upper quadrant of the abdomen; multiple abscesses (actinomycotic?) of lung; bilateral paravertebral and psoas abscesses; subcutaneous abscess of the right lateral wall of the chest; old adhesive pericarditis; fistulas of the right lower quadrant and right wall of the chest posteriorly; focal interstitial pyelonephritis.

Histologic examination of the lungs revealed only a rare colony of organisms resembling Actinomyces. These colonies, except for the absence of eosinophilic clubs, appeared fairly typical of *Actinomyces bovis* when stained with hematoxylin and eosin. By use of the Gram stain, however, it was noted that the gram-positive organisms were rather short and thick without definite branching filaments. They were not acid-fast. Surrounding the abscesses there was an organizing fibrinopurulent pneumonia but no definite connective tissue wall, lipophages nor giant cells (Fig. 2).

FIG. 1. Section of ovary showing much fibrosis and a sulfur granule in an abscess typical of *Actinomyces bovis* infection. × 42.

FIG. 2. Section of lung in an equivocal case showing very little fibrosis around the abscess. Filaments suggestive of *Actinomyces bovis* were present. × 100.

FIG. 3. Histologic reaction simulating actinomycosis. Note fibrotic wall around an abscess containing an eosinophilic mass that resembles a colony of *Actinomyces bovis*. No filaments could be found by the Gram stain. × 85.

FIG. 4. Small abscesses with organizing pneumonia in a case of pulmonary nocardiosis. × 85.









FIGS. 1–4

Histologic sections of the psoas muscle revealed much chronic suppurative inflammation with atrophy and fibrosis of the muscle cells, but no organisms were observed.

In sections of both kidneys there were foci of suppurative interstitial nephritis (pyelonephritis), but no organisms were observed.

In conclusion, because we were limited to histologic studies we could not decide definitely whether this was or was not a *bona fide* case of actinomycosis.

CASE APPARENTLY DUE TO SOME OTHER ETIOLOGIC AGENT BUT SIMULATING ACTINOMYCOSIS

Case 3

The patient was a woman aged 39. Three and one-half years previously, pelvic infection had developed after use of a stem pessary and five pelvic operations had been performed for drainage of an abscess of the broad ligament. For three years there had been constant drainage of the abdominal wound with recurring attacks of chills, fever, loss of weight and weakness. The results of repeated examination of exudate of the sinus were negative for tubercle bacilli and sulfur granules. Operation was performed for tuboovarian abscesses and closure of the enterocutaneous fistula; the patient died 50 days after operation. At necropsy the following anatomic diagnoses were made: Recent (thirteen day) resections of two ribs (open operation) for multilocular abscesses of left lung with extension to pleural cavity; healed duodenal ulcer; severe coronary arteriosclerosis with chronic infarction of the apex of the left ventricle; chronic ulcerative cholecystitis.

Histologic examination of the left lung revealed numerous abscesses containing atypical colonies of organisms with eosinophilic clubs. No filaments could be demonstrated by use of the Brown-Gram stain. Surrounding the abscesses there was a poorly developed wall of connective tissue consisting largely of fibroblasts (Fig. 3). There was an organizing pneumonia in the adjacent lung and many of the alveoli were filled with lipophages.

This appears to be a case of nonspecific infection in which the histologic appearance of the lesion and the organisms simulate those of actinomycosis.

NOCARDIOSIS

Case 4

The patient, a boy aged 6, had chest pain, cough, yellowish green blood-streaked sputum, a temperature of 99 to 105 F. and loss of weight for three months. He was treated with penicillin daily without improvement and was given an occasional dose of sulfonamide for a day at a time. Leukocytes numbered 28,000 per cubic millimeter of blood. The sedimentation rate (Westergren) was 69 mm. in one hour. Cultures and smears were negative for acid-fast bacilli. The child died five days after admission to the hospital. At necropsy the following anatomic diagnoses were made: Multiple actinomycotic abscesses of the lungs with respiratory insufficiency (clinical); organizing pneumonia.

Histologic examination of the lungs revealed multiple abscesses with much necrosis of tissue and practically no connective tissue capsule (Fig. 4). Large colonies of organisms could not be found, but by use of the Brown-Gram stain, isolated filaments were observed in the pus. In the pulmonary parenchyma adjacent to the abscesses there were patches of

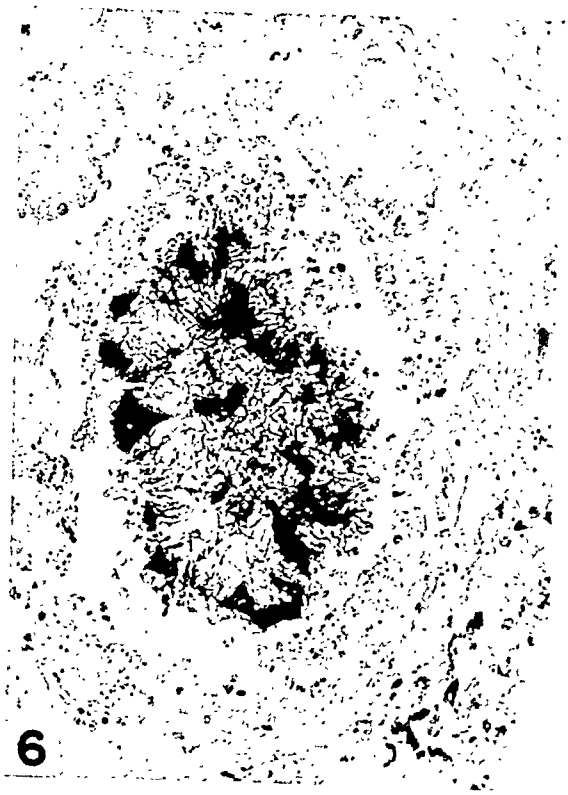
FIG. 5. Sulfur granule with only a few filaments and many clubs. There is, however, no direct relationship between the two structures. × 500.

FIG. 6. Sulfur granule with very few clubs but with many bacteria other than *Actinomyces bovis*. × 235.

FIG. 7. Sulfur granules with amorphous pink-staining mass in center, with filaments but without clubs at the periphery. Some filaments are stained gram-positive, others gram-negative. Note the masses of cocci present as mixed infection. × 500.

FIG. 8. Granule with much pink-staining material throughout the mass, with only a few filaments. Presumably this is *Actinomyces bovis*. × 500.









FIGS. 5-8

organizing fibrinopurulent exudate. Many of the alveoli were filled with phagocytes, some of which contained lipoids. In some sections there were regions of necrosis with Langhans type of giant cells at the periphery, suggestive of a tuberculous process. No tubercle bacilli could be found, however.

COMMENT

Pathologic features. In conformity with the experience of other workers the fatal cases of actinomycosis caused by *Actinomyces bovis* comprising this series could be classified into three broad groups: cervicofacial, thoracic and abdominal. In contrast to most clinical reports the incidence of the cervicofacial type was the lowest (4 cases). This is to be expected since our report deals only with fatal cases and the cervicofacial variety is most amenable to treatment. There were 5 cases of the thoracic type and 12 cases of the abdominal type. In 7 cases both thoracic and abdominal viscera were involved at the time of death. Appendectomy preceded or followed the onset of symptoms in 8 of the cases with predominant involvement of the abdominal viscera.

Wherever the lesions occurred, suppuration, sinus formation and scarring were observed. Burrowing of the inflammatory process was characteristically present and led frequently to the production of intercommunicating abscesses. Frequently the process was healing in one location with active progression in other sites. Grossly the visceral lesions were similar to a variety of other granulomas. The presence of suppuration and sinus formation with an extensive fibrous tissue reaction and a yellow color were the most constant gross characteristics.

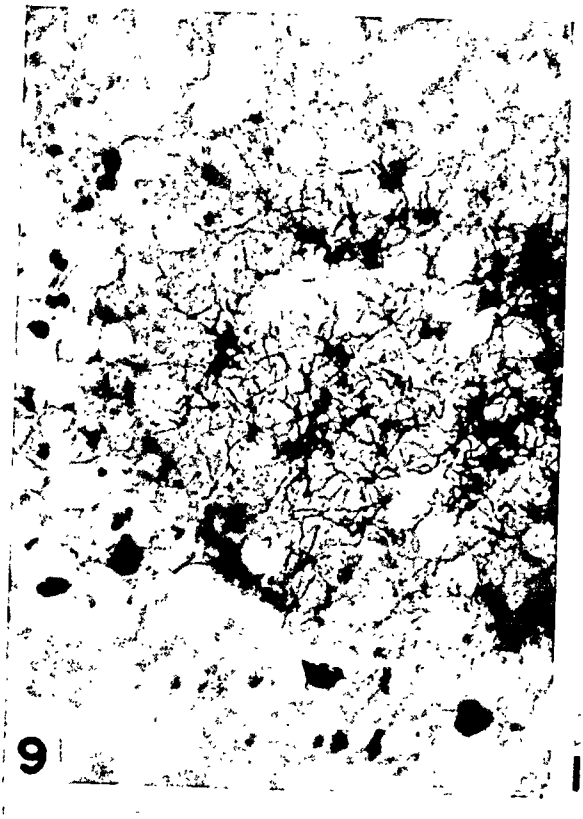
Histologically, suppuration was a feature of the active lesions in all of these cases. The abscesses varied considerably in size, and frequently there were intercommunication and coalescence. The appearance and the amount of granulation tissue around the abscesses varied considerably in the different cases. In 4 cases there was a minimal amount of fibrous tissue in the walls of the abscesses but in most of the others fibrosis was large in amount, and in some of the lesions suppuration was absent and considerable healing (scarring) was apparent. Included in the granulation tissue about the abscess were variable numbers of polymorphonuclear cells, plasma cells and lipoid histiocytes and sometimes the Langhans' type of giant cells. The latter were also occasionally observed in the abscess proper. Phagocytes containing lipoid were often prominent and were responsible for the yellow gross appearance of the lesions. The number of fibroblasts and collagen fibers in the wall varied considerably with the organ in which the lesion was found. This connective tissue reaction was minimal in lesions of the central nervous system, often slight in the lungs and most marked in the liver, kidneys, ovaries and subcutaneous or muscular tissue. The rela-

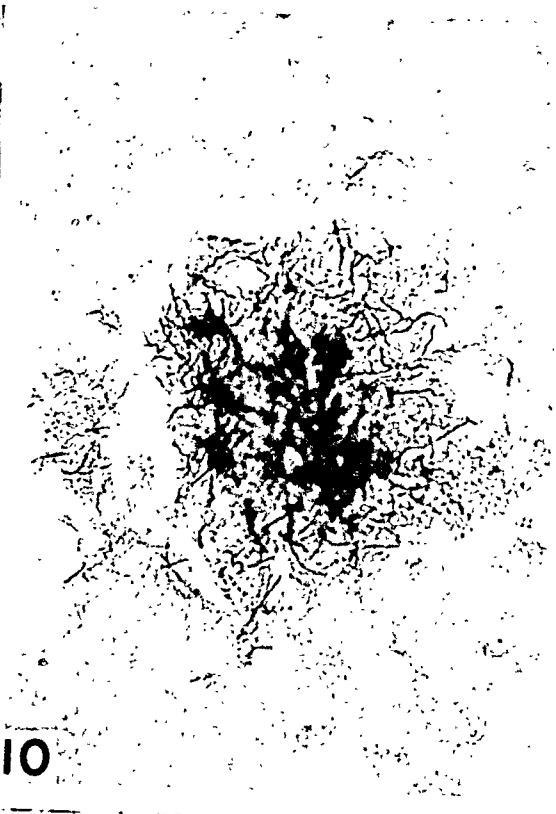
FIG. 9. Small colony with tangled mass of filaments, possibly *Actinomyces bovis*, but not forming a compact granule. Note both gram-positive and gram-negative filaments. × 500.

FIG. 10. Similar but smaller colonies, very few filaments. × 500. From same case as shown in Figures 7 and 11.

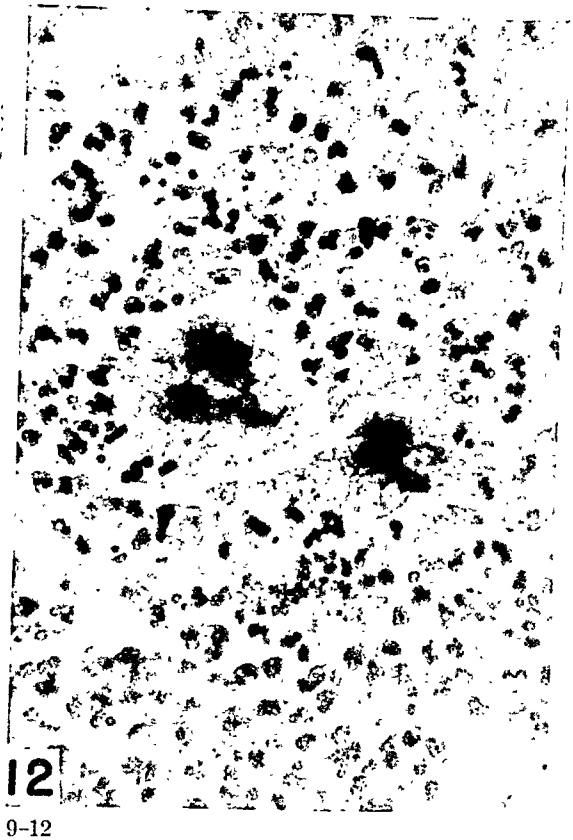
FIG. 11. Similar but small colonies, very few filaments but many cocci. × 500. From same case as shown in Figure 7 and 10.

FIG. 12. Very few filaments plus some organisms resembling diphtheroids. × 500.









FIGS. 9–12

tively slight resistance of the brain to actinomycotic infection is consistent with its behavior in other infections and also in noninfected wounds.

Worthy of special comment is the fact that the equivocal cases in this series and those cases with lesions simulating actinomycosis but caused by other bacteria could not be separated on a histologic basis from cases of actinomycosis which had been proved bacteriologically. Histologically, these cases presented an appearance consistent with a diagnosis of *bona fide* actinomycosis. This again emphasizes the absolute necessity of obtaining bacteriologic verification in every case of suspected *Actinomyces bovis* infection.

Also worthy of special comment is the fact that the 2 cases of Nocardia infection (only one proved by culture) presented lesions strikingly different from those resulting from *Actinomyces bovis*. In each case the histologic picture was dominated in most of the lesions by rapidly progressing necrosis of tissue without evidence of a granulation tissue wall. In one of these cases, colonies of organisms consisting of gram-positive branching filaments without clubbing were observed in the necrotic lesions. In the other case only isolated organisms in phagocytic cells were observed, although the cultures revealed large numbers of colonies in pure culture. In this latter case there were also numerous lesions consisting of a central region of caseous necrosis with a surrounding zone of granulation tissue consisting of numerous fibroblasts and occasional lymphocytes, plasma cells and polymorphonuclear leukocytes. A few phagocytes containing lipoid and numerous giant cells of the Langhans type were also observed in the granulation tissue. The appearance superficially resembled tuberculous lesions.

From the data available, using only histologic material and special stains, organisms other than *Actinomyces bovis* were demonstrated in 13 of 21 cases. Since it is frequently difficult to demonstrate bacteria in tissues by staining procedures, it is highly probable that nearly all the cases studied could have been shown to have mixed infection had it been possible to employ cultural procedures. This correlates well with the difficulty experienced in isolating *Actinomyces bovis* in pure culture from clinical cases. In our experience this organism is found in apparently pure culture in only an occasional patient.

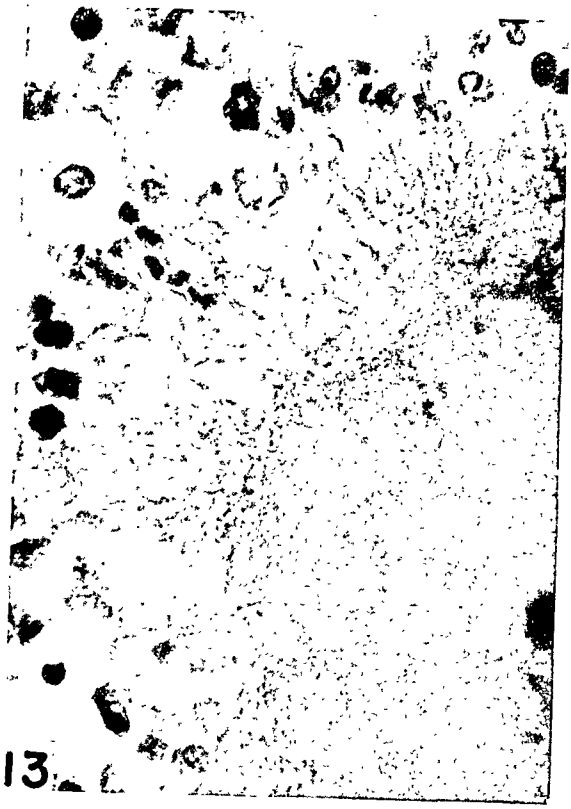
Bacteriologic features. The occurrence of nonspecific infections in various organs in these cases supports the evidence that most, if not all, infections with *Actinomyces bovis* in man are mixed infections. A number of the brain abscesses, for example, were not actinomycotic in origin but the result of bacterial invasion, presumably metastatic through the blood stream. The high incidence of interstitial nephritis (pyelonephritis) in these cases is especially convincing. In 12

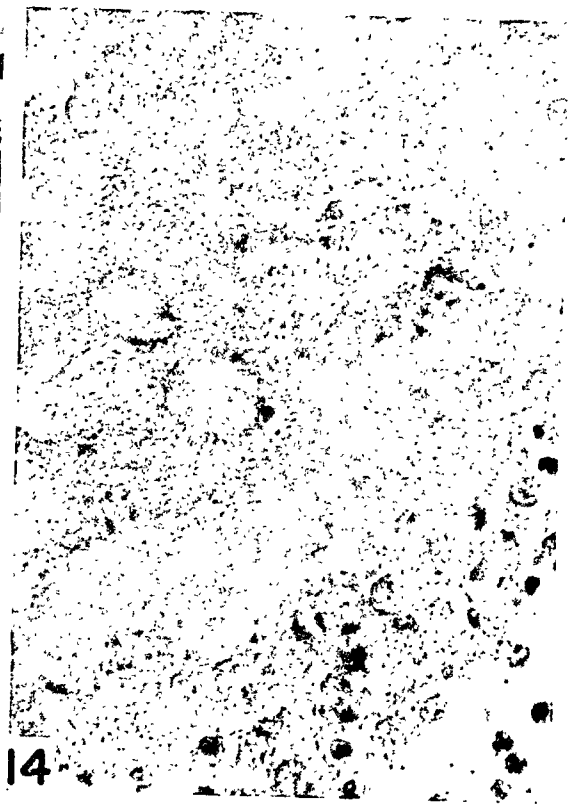
FIG. 13. Material in brain abscess resembling sulfur granule in hematoxylin and eosin stain with absence of filaments by Brown-Gram stain. Cultures and direct examination negative for *Actinomyces bovis* but positive for Streptococcus, presumably metastatic from lesion in lung due to *Actinomyces bovis*. Lung culture positive for *Actinomyces bovis*. × 1000.

FIG. 14. Granules simulating *Actinomyces bovis* in an abscess but no filaments are present. × 1000. From same case as shown in Figure 3.

FIG. 15. Pink-staining granule resembling *Actinomyces bovis* in an abscess but showing no filaments. × 500.

FIG. 16. Gram stain showing a colony of streptococci which resembled *Actinomyces bovis* in hematoxylin and eosin stain. × 1000.


13


14


15


16

FIGS. 13–16

of the 16 cases of *Actinomyces bovis* infections in which sections of a kidney were available for study, interstitial, nonspecific infection of one or both kidneys was present. As in the case of the abscesses of the brain these renal infections were presumably the result of invasion of the blood stream by bacteria present in the actinomycotic lesions.

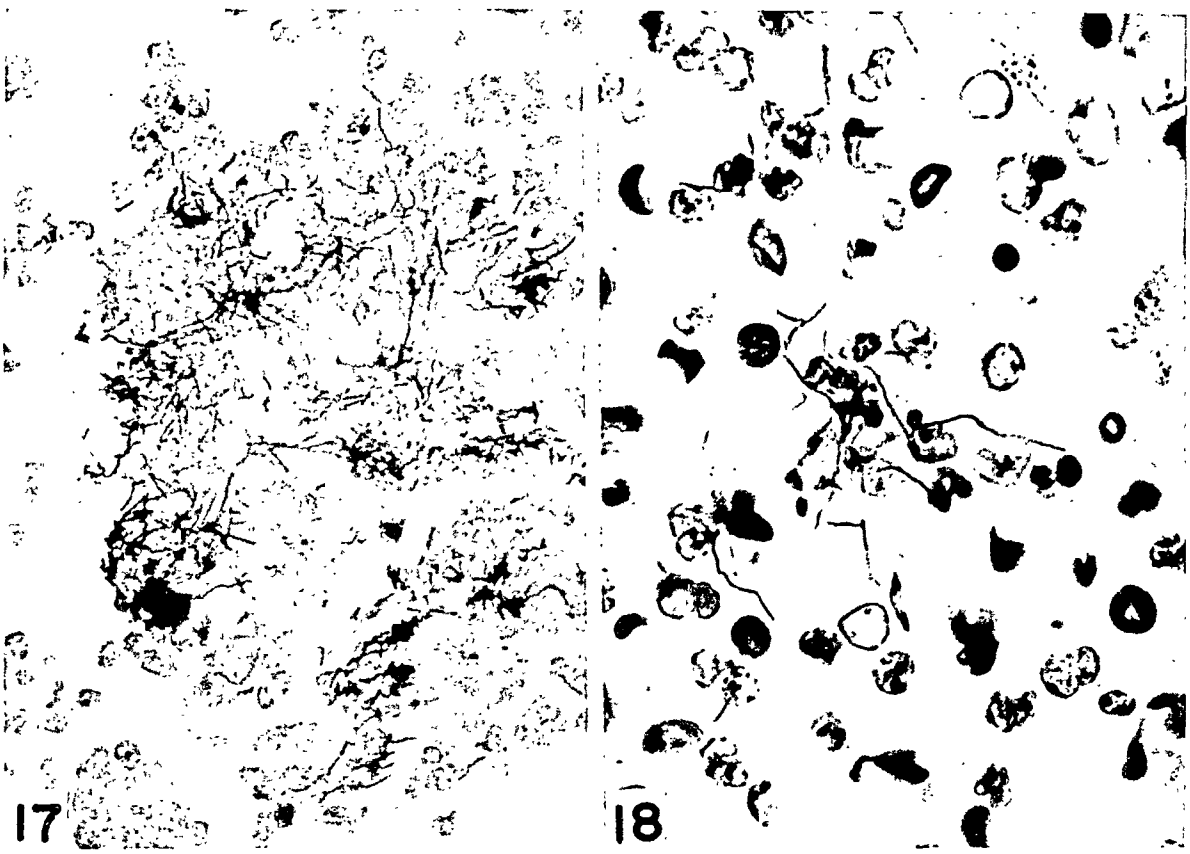


FIG. 17. Ill-defined mass of filaments (gram-positive and gram-negative) of *Actinomyces bovis* resembling Nocardia. × 500. Same case as shown in Figure 6.
FIG. 18. Filaments without formation of sulfur granules, resembling Nocardia. × 1000.

Mixed infection is to be expected if the process results from the introduction of material from the gastrointestinal tract or the upper part of the respiratory tract. Likewise, the commonly encountered contaminants such as staphylococci, streptococci, Proteus, *Escherichia coli* and diphtheroids, being adapted to the animal environment, are able to persist in the necrotic regions as soon as they are introduced. It may well be that in some cases such contaminants help establish or maintain the pathologic process, or both establish and maintain it, as Israël apparently believed. Kay has also questioned whether the associated organisms might not be clinically important in cases of pulmonary actinomycosis. On the other hand, when infections are due to Nocardia (the aerobic type), the organism, being derived from sources outside the body, carries with it saprophytes which cannot easily adapt to the usual defenses of the animal body and the well-

developed pathologic process is then likely to contain but the single species. If, however, draining sinuses have developed and persisted for any great length of time, mixed infection is likely to be encountered as a result of outside contamination. While mixed infection was common in the cases studied for this presentation, one can only speculate in the absence of cultures as to the relation of the associated organisms to such complications as pyelonephritis. It is highly probable, however, that secondary invaders were responsible for the brain lesions in some cases since actinomycetes could not be demonstrated in the tissue. Further studies on clinical cases will be necessary to determine whether the associated organisms influence the response to treatment. It is known that *Actinomyces bovis* is sensitive to penicillin *in vitro* and clinical data suggest its use *in vivo*. It is unlikely that strains of *Actinomyces bovis* ever develop resistance to penicillin during a course of treatment, since we have shown by *in vitro* experiments that strains adapt themselves very slowly if at all to the antibiotic (unpublished data).

Not only are the sulfur granules of *Actinomyces bovis* likely to contain other micro-organisms, but other bacteria may frequently produce structures which grossly and by superficial microscopic examination simulate the former. This has been emphasized many times by other observers.[3, 15, 23] Occasionally structures resembling sulfur granules may be encountered in which no micro-organism can be demonstrated by stains. Kimball and Frank reported the finding of *Actinomyces bovis* in smooth form (without granules) in fistulous withers of horses. If this be true the diagnosis of actinomycosis can be made with certainty only by growing the organism and proving that it is micro-aerophilic and produces branching filaments. However, frequently, because of the difficulty from a practical clinical point of view of isolating the organism in pure culture even from sulfur granules, one has to be content with morphologic studies. However, these should always include a Gram stain of the sulfur granule, no matter how typical the clinical course, the histologic pattern of the biopsy (or necropsy) specimen or the gross and microscopic appearance of the granule itself. In cases of nocardiosis, in our limited experience and according to the literature, granules are not commonly produced; but if they are encountered, they are usually much smaller and softer than those associated with *Actinomyces bovis*. Finally, if the granule is produced by Nocardia, the organism can be easily isolated on many mediums under aerobic conditions. This emphasizes the importance of attempting cultures in all cases.

From the 21 cases studied, considerable variations were encountered in the size, staining reaction and internal structure of the so-called sulfur granules (Figs. 5–18, Brown-Gram technic). Indeed, in some of these, it was impossible to decide whether the organisms were or were not *Actinomyces bovis*. The filaments sometimes stained red, sometimes blue and sometimes they were mixed red and blue, even in a given colony. Sometimes the filaments were so numerous that they formed thick, tangled, dense masses, and sometimes they were few though the area covered by the granule was fairly large. Sometimes clubs were present, sometimes they were not; when they were present they did

not appear to have any relationship to the tips of the filaments. Sometimes the organisms were so short that they could hardly be called filaments and more closely resembled diphtheroids. Whether they represented anaerobic diphtheroids (which are common even in normal tissues) which resemble the so-called smooth form of *Actinomyces bovis* or whether they actually were the latter, cannot be positively stated in the absence of culture studies. In the current clinical material we have never been able to demonstrate or recognize such smooth forms, although we have encountered many cases with anaerobic diphtheroids resembling the descriptions of them. We have not had occasion to examine material from fistulous withers of horses. Sulfur granules could not be demonstrated in the case of nocardiosis which was proved by cultures. A second case, presumed to be nocardiosis on morphologic grounds, likewise had no demonstrable sulfur granules. In this case the organisms were distributed diffusely throughout the lesions. However, some tissues showed such loose arrangement of the filaments, but with regions suggesting clumps, that it was impossible to determine for certain whether they should or should not be referred to as "sulfur granules". It, therefore, becomes difficult in certain cases, on the basis of histologic information alone, to determine whether the filaments are those of Nocardia or *Actinomyces bovis*. It becomes of primary importance, however, in scientific modern medicine, that the infections referred to clinically as actinomycosis, be studied thoroughly bacteriologically to determine as precisely as possible the nature of the causative organism, for this will determine the types of treatment that the patient is to receive.

Organisms of the genus Nocardia which are likely to be associated with human infections have been shown by *in vitro* tests to be fairly sensitive to sulfonamides, and clinical cures of such infections treated with sulfonamides have been reported.[4, 6, 7, 14, 19, 22, 32] On the other hand, *Actinomyces bovis* has been shown by *in vitro* experiments[11] to be very sensitive to penicillin, and this has been found to be the drug of choice in treating infections associated with this organism.[2, 8, 16, 17, 28, 30]

Numerous cases of infection reported in the literature as being caused by "aerobic actinomyces" have shown certain clinical and postmortem features which serve to differentiate the disease from that associated with the anaerobic *Actinomyces bovis*. The abscesses have occurred in widely separated parts of the body (for example, extremities, kidneys, liver and lungs), suggesting widespread hematogenous dissemination. Frequently there is extensive involvement of lymph nodes with much suppuration but very little fibrosis. *Actinomyces bovis*, on the other hand, seldom if ever gives rise to extensive hematogenous dissemination* and nearly always stimulates the production of marked fibrosis. Localized processes may occur, for example, in mycetoma where it would be impossible on the basis of clinical features alone to state the probable nature of the organism. Because of the fundamental differences in the biologic nature of the organisms,

* In many cases cited in the literature as being "generalized actinomycosis" the organisms were *not* of the anaerobic *Actinomyces bovis* type, but were aerobic. Some of these have been called Nocardia by the authors, the other cases probably were caused by Nocardia as judged by the clinical and pathologic findings.[13] In other cases the bacteriologic data are too meager to permit adequate evaluation.[21]

the differences in their clinical response to chemotherapeutic agents and their different clinical manifestations and pathologic processes, it is urged that the suggestions which have already been made[14] be given closer attention by clinical pathologists and others responsible for making diagnoses; namely, that the terms "Actinomyces" and "actinomycosis" be used respectively when referring to the anaerobic or micro-aerophilic organism or the disease associated with it, and that the terms "Nocardia" and "nocardiosis" be used when referring to the aerobic organism or the disease associated with it. This division of names should materially aid the clinician responsible for treating the patient to keep clearly in mind the preferred drug for a given case. Incidentally it would make for a more scientific approach to medicine as applied in these cases.

CONCLUSIONS

1. Of 35 cases which were designated actinomycosis at necropsy, the organism, *Actinomyces bovis*, could be demonstrated in histologic sections with reasonable certainty in only 21, with considerable doubt in 3, and not at all in 11. Two of the 11 cases had markedly different gross and histologic features, and were suggestive of nocardiosis. Nocardia was isolated in pure culture from one of these.

2. In at least 13 of the 21 cases showing *Actinomyces bovis*, it was possible to demonstrate histologic evidence of mixed infection.

3. The probable clinical significance of the organisms associated with *Actinomyces bovis* was reflected in the presence of renal involvement in 12 of 16 cases (pyelonephritis and abscess formation) and nonactinomycotic brain abscesses in 5 cases.

4. Based on the study of fixed tissues, the evidence obtained indicated considerable variation in the detailed structure of colonies presumed to be *Actinomyces bovis*. The variations included presence or absence of clubs, apparent fusion of clubs, staining reaction of clubs by the Brown-Gram technic, number of filaments, length of filaments (some resembling diphtheroids) and compactness of the colonies (some having almost a diffuse growth). The sections also showed considerable variations in the amount of fibrosis.

5. The presence of sulfur granules and the histologic pattern, usually considered characteristic of actinomycosis, may be produced by organisms other than *Actinomyces bovis*. In many cases culture procedures may be necessary to make the differentiation.

6. Much of the confusion concerning the diagnosis and treatment of what has heretofore been called "actinomycosis" may be readily removed if thorough bacteriologic studies are made of each case, and if one uses the terms "Nocardia" for the aerobic form of the organism and "nocardiosis" for the resulting disease process. The term "actinomycosis" should be reserved for the condition asosciated with the anaerobic organism designated by Bergey's Manual as *Actinomyces bovis*.

REFERENCES

1. Abrahams, Irving, and Miller, J. K.: The in vitro action of sulfonamides and penicillin on actinomyces. J. Bact., **51**: 145–148, 1946.
2. Altemeier, W. A., and Wadsworth, C. L.: Penicillin, its use in surgery and influence on earlier types of chemotherapy. Surg., Gynec. and Obst., **84**: 540–552, 1947.

3. AUGER, CARLTON: Human actinobacillary and staphylococcic actinophytosis. Am. J. Clin. Path., **18:** 645–652, 1948.
4. BENBOW, E., SMITH, D., AND GRIMSON, K.: Sulfonamide therapy in actinomycosis; two cases caused by aerobic partially acid-fast actinomyces. Am. Rev. Tuberc., **49:** 395–407, 1944.
5. BIBBY, B. G., AND KNIGHTON, H. T.: The actinomyces of the human mouth. J. Infect. Dis., **69:** 148–154, 1941.
6. BILLINGTON, R. W.: Actinomycosis treated with sulphapyridine. Brit. M. J., **1:** 326, 1944.
7. BINFORD, C. H., AND LANE, J. D.: Actinomycosis due to *Nocardia asteroides*; report of a case. Am. J. Clin. Path., **15:** 17–23, 1945.
8. BOHAN, E. M., AND SERINO, G. S.: Cervicofacial actinomycosis successfully treated by penicillin and repeated aspirations. Delaware State M. J., **17:** 213–214, 1945.
9. BOLLINGER, O.: Quoted by Cope, Zachary. Actinomycosis. New York: Oxford University Press, 248 pp., 1938.
10. BOSTROEM: Untersuchungen über die Aktinomykose des Menschen. Beitr. z. path. Anat. u. z. allg. Path., **9:** 1–240, 1890.
11. DRAKE, C. H.: The action of penicillin on several genera of Actinomycetales. J. Bact., **51:** 199–203, 1946.
12. EMMONS, C. W.: The isolation of *Actinomyces bovis* from tonsillar granules. Pub. Health Rep., **53:** 1967–1975, 1938.
13. EPPINGER, HANS: Ueber eine neue, pathogene Cladothrix und eine durch Sie hervorgerufene Pseudotuberculosis (cladothrichica). Beitr. z. path. Anat. u. z. allg. Path., **9:** 287–328, 1891.
14. GLOVER, R. P., HERRELL, W. E., HEILMAN, F. R., AND PFUETZE, K. H.: Nocardiosis, *Nocardia asteroides* infection simulating pulmonary tuberculosis. J.A.M.A., **136:** 172–175, 1948.
15. HAGAN, W. A.: The Infectious Diseases of Domestic Animals with Special Reference to Etiology, Diagnosis, and Biologic Therapy. Ithaca, New York: Comstock Publishing Company, 665 pp., 1943.
16. HAMILTON, A. J. C., AND KIRKPATRICK, H. J. R.: Actinomycosis successfully treated with penicillin; report of 2 cases. Brit. M. J., **2:** 728, 1945.
17. HENDRICKSON, G. G., AND LEHMAN, E. P.: Cervicofacial actinomycosis successfully treated by penicillin without surgical drainage. J.A.M.A., **128:** 438, 1945.
18. ISRAËL, JAMES: Neue Beobachtungen auf dem Gebiete der Mykosen des Menschen. Virchows Arch. f. path. Anat., **74:** 15–53, 1878.
19. JACOBSON, J. R., AND CLOWARD, R. B.: Actinomycosis of the central nervous system. J.A.M.A., **137:** 769–771, 1948.
20. KAY, E. B.: Bronchopulmonary actinomycosis. Ann. Int. Med., **26:** 581–593, 1947.
21. KIMBALL, ALICE, AND FRANK, E. R.: The isolation of *Actinomyces bovis* from fistulous withers and poll evil. Am. J. Vet. Research, **6:** 39–44, 1945.
22. LYONS, CHAMP, OWEN, CORA R., AND AYERS, W. B.: Sulfonamide therapy in actinomycotic infections. Surgery, **14:** 99–104, 1943.
23. MOORE, MORRIS: Radiate formation on pathogenic fungi in human tissue. Arch. Path., **42:** 113-153, 1946.
24. MUSKATBLIT, EMANUEL: Primary actinomycosis of the skin; report of a case. Arch. Dermat. and Syph., **56:** 706–712, 1947.
25. NAESLUND, CARL: Studies of Actinomyces from the oral cavity. Acta path. et microbiol. Scandinav., **2:** 110–140, 1925.
26. NAESLUND, CARL: Studies of tartar formation. Acta path. et microbiol. Scandinav., **3:** 637–680, 1926.
27. NAESLUND, CARL: Experimentelle Übertragung der Aktinomykose vom Menschen auf Versuchstiere mittels Reinkulturen der Actinomycesform. Wolff-Israël (Act. Israëli). Acta path. et microbiol. Scandinav., **6:** 66–77, 1929.
28. NICHOLS, D. R., AND HERRELL, W. E.: Penicillin in the treatment of actinomycosis. J. Lab. and Clin. Med., **33:** 521–525, 1948.
29. PONFICK: Quoted by Cope, Zachary. Actinomycosis. New York: Oxford University Press, 248 pp., 1938.
30. ROBERTS, J. E. H., TUBBS, O. S., AND BATES, MICHAEL: Pleural and pulmonary suppuration treated with penicillin. Lancet, **1:** 39–45, 1945.
31. ROSEBURY, T.: The parasitic actinomycetes and other filamentous micro-organisms of the mouth; review of their characteristics and relationships, of bacteriology of actinomycosis, and of salivary calculus in man. Bact. Rev., **8:** 189–223, 1944.
32. SHAW, F. W., HOLT, REBECCA, A., AND RAY, E. S.: Pulmonary actinomycosis due to *Actinomyces asteroides*. Virginia M. Monthly, **73:** 362–368, 1946.
33. WOLFF, MAX, AND ISRAËL, JAMES: Ueber Reincultur des Actinomyces und seine Uebertragbarkeit auf Thiere. Virchows Arch. f. path. Anat., **126:** 11–59, 1891.
34. WRIGHT, J. H.: The biology of the micro-organisms of actinomycosis. J. Med. Res., **13:** 349–404, 1905.

A SIMPLE DIRECT METHOD FOR THE ELECTRON MICROSCOPY OF PERIPHERAL BLOOD CELLS*

JOHN W. REBUCK, M.D.†

From the Laboratories of the Henry Ford Hospital, Detroit, Michigan

The need of the hematologist for methods applicable to the study of the cells of the peripheral blood with the electron microscope has been pressing. Almost a decade ago Wolpers and Ruska[36] studied thrombocyte structure in relation to fibrin deposition. Wolpers,[35] separately, devised a technic for the direct electron micrography of red blood corpuscles and their membranes. Replica or indirect studies of the red blood corpuscular surfaces were reported later by Barnes, Burton and Scott[1] and by Jones.[17]

More recent direct observations include those of Claude and Fullam[6] who, with the electron microscope, studied mitochondria isolated from lymphosarcoma cells of the rat. They brought evidence for an enveloping membrane as well as for small elements within the body of certain mitochondria. Reed and Reed[27] studied erythrocytes from dog's blood with reference to patterned aggregates of hemoglobin and other components which varied with the rapidity of drying of the erythrocyte *in vacuo*. Fine granules, rough, fibroblast-like forms, and massed aggregates arising from coalescence of the smaller structures were observed. Hovanitz[14] studied "resting" chromosomes obtained from nuclei of erythrocytes of the chicken. A definite spiral structure was present which may have represented a thread imbedded in a matrix. Appendages were seen with their attachment threads. When the chromosomes were dissolved in *M* NaCl and precipitated, anastomosing strands composed of minute particles about 50 millimicrons in diameter were produced.

Claude, Porter and Pickels[7] were able to obtain macrophages from tissue cultures of centrifuged blood of normal chickens after explanting small fragments of the buffy coat. Their micrograph (their Fig. 1) depicted only the cytoplasm of such a macrophage. Golgi bodies, mitochondria and the particulate components (microsomes) of the ground substance were well portrayed.

Bessis and Burstein[4, 5] studied the progression of structural changes occurring in thrombocytes *in vitro*. After addition of anticoagulant, the blood was centrifuged and a drop of the supernatant fluid rich in thrombocytes was utilized for electron microscopy. Their numerous excellent micrographs depict the succeeding stages, classified as circulating forms, dendritic forms, transitional forms, and ultimately, expanded forms. Expansion of the platelet is preceded by pseudopodia formation. In a later series of investigations, Bessis[2, 3] employed the shadow-casting technic and demonstrated that thrombocyte protoplasm presented numerous radiate fibrils and rarer arcuate forms. The fibrils were composed of small spherules (50 to 200 millimicrons in diameter) separated from one

* Received for publication, November 12, 1948.

† With the technical assistance of Miss Helen L. Woods and Miss E. A. Monaghan.

another by varying intervals. The pseudopodia of the dendritic stage contained one or more fibrils. Round, clear areas containing a single central spherule were observed in the substance of the thrombocyte. The spherules present in the thrombocyte protoplasm were also disseminated in the medium surrounding the thrombocytes.

Rebuck and Woods[23, 24, 25] reported on several technics for the electron microscopic study of the various forms of granulocytes and lymphocytes and their precursors which had been obtained from the hematopoietic organs and of the granulocytes and histiocytes (macrophages) which had been obtained from inflammatory exudates of man. In the histiocytes a fibrillar structural meshwork could be made out in both the nuclear and cytoplasmic protoplasm. Some of the fibrils measured as little as 140 Angström's units in breadth. The nuclear protoplasmic interstices formed by such fibrils were smaller than the corresponding cytoplasmic areas. The nuclear membrane appeared as the common membrane of attachment for both cytoplasmic and nuclear fibrillar strands. Neutrophil granules, depicted as definitive structures for the first time, appeared as round, oval or rodlike forms which exhibited surprisingly very little resistance to the passage of electrons. Later, Rebuck, Woods and Monaghan[26] modified the Beck-Hertz technic to permit direct mounting of sickle cells for electron microscopy. Such micrographs allowed better appreciation of the extent and structure of polar filaments in sickle cells and also introduced an interesting field of study in this disease with visualization of hemoglobin-corpuscular membrane relationships.

MATERIALS AND METHODS

Peripheral blood was obtained by venipuncture. The specimen was placed at once in a paraffin-lined test tube containing a small amount of powdered heparin and gently inverted to mix the blood and the anticoagulant. The blood specimen was then placed in a Wintrobe hematocrit tube with a capillary pipet and centrifuged for a short time until the four major layers (plasma, thrombocytes, nucleated cells and erythrocytes) had settled, at least macroscopically. In dealing with patients with normal or low leukocytic counts, the necessity for preliminary concentration of several tubes of blood may present itself in order to obtain a final concentrated column of the leukocytic layer of sufficient height for selective sampling.

The plasma and thrombocyte layers were withdrawn from the centrifuged specimen by means of a capillary pipet. If desired, either layer can, of course, be studied in respect to thrombocyte studies in the manner to be described below for the nucleated-cell layer or by the methods recently utilized by Bessis and Burstein[4, 5] and Bessis.[2, 3]

The layer of nucleated cells was next carefully withdrawn and expressed, drop by drop, onto successive, separate, formvar-covered glass slides. Each drop was spread over the formvar-covered surface with an ordinary glass slide in a manner similar to that employed in making blood smears for study with the light microscope. It is important that the films be exceedingly thin. The thin films of concentrated leukocyte layer were air-dried and fixed. Although a thin layer of plasma was in all likelihood still adherent to the nucleated cells, it was of such low density that it did not interfere seriously with structural studies. Fixation and washing probably still further decreased the density of the ever present blood plasma film coating the nucleated cells.

Fixation was accomplished by immersion of the entire film-covered glass slide, specimen side upwards, in 2 per cent osmic acid solution in water for from 14.5 to 23.5 hours, followed



All figures reduced to $\frac{4}{5}$ of original size.

FIG. 1. Electron micrograph of early dendritic stages of thrombocytes from normal blood. × 4800.

FIG. 2. Electron micrograph of dendritic stages of thrombocytes from normal blood. × 4800.

FIG. 3. Electron micrograph of segmented neutrophilic leukocyte from normal blood. × 4800.

FIG. 4. Electron micrograph of small lymphocyte from normal blood. × 4800.

FIG. 5. Electron micrograph of medium-sized lymphocyte from normal blood. × 4800.

FIG. 6. Electron micrograph of a small lymphocyte from the blood in chronic lymphocytic leukemia. × 4800.

by rinsing for from 10 to 35 minutes in distilled water. This fixation method was suggested originally by Claude, Porter and Pickels[7] and was utilized by Porter and Thompson.[22]

The final specimen mounting was rapidly effected in the manner reported by Rebuck and Woods.[24] This is a direct method. The resultant specimens are not replicas. The method consisted of: (1) selection of a suitable cell group under the light microscope; (2) placement of a specimen screen over the selected cell group; (3) destruction of the thickened end of formvar film at the end of the slide by means of a scalpel, thus loosening the formvar film at one edge; (4) covering of entire formvar film including the screen-covered areas with Scotch tape; (5) removal of the Scotch tape and the now adherent formvar film with interposed specimen screen; (6) sharp dissection of the formvar film about the screen circumference and removal of the screen from the Scotch tape, the cells for study now being trapped between the specimen screen and the formvar film.

Figures 1 to 6 and Figures 8 to 12 are all electron micrographs of peripheral blood cells or elements prepared in the above described manner. Figure 7, however, depicts a bizarre red corpuscular form obtained from a case of sickle cell anemia. This latter cell was prepared in the manner described by Rebuck, Woods and Monaghan[26] and is included for purposes of comparison of the background-contrast obtained by the two different technics. A micron scale has been affixed to each of the micrographs.

The author's[24] modification of the Altmann-Gersh technic of quick-freezing and dehydration of leukocytes may, of course, be substituted for the steps involving osmic acid fixation outlined above. From previous studies nuclear and specific granular structure can be obtained in great detail in cells which have received such preparatory treatment. Inasmuch as such a modification of technic requires additional complex apparatus and material, it is not the subject of the present report.

The nomenclature employed is that recommended in the first report of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs.[12]

RESULTS

Figures 1 and 2 are electron micrographs (× 4800; reduced to four-fifths of original magnification of 6000) of several circulating thrombocytes of man. The thrombocyte at the right in Figure 1 is just emerging from the structure of the circulating form, the thrombocyte at the left presents several pseudopodia at the dendritic stage of its transformation. The pseudopodia of the dendritic stage are well represented in the central thrombocyte of Figure 2. The aggregation of granules, which possesses an affinity for the osmic acid, is striking in the granulomere of the depicted stages of transformation. Similar results in thrombocytic studies have been presented by Wolpers and Ruska,[36] Bessis and Burstein[4, 5] and Bessis.[2, 3]

Figure 3 is an electron micrograph (× 4800) of a normal segmented neutrophilic leukocyte of man. The lobular membranes and chromatin pattern are distinct. The specific granules are poorly delineated by the method of fixation employed. Electron micrographs of neutrophil granules have been presented in an earlier work[24] to which the reader is referred.

Figure 4 is an electron micrograph (× 4800) of an ordinary small lymphocyte

from the normal peripheral blood of man. At this magnification the nuclear structural pattern reveals the same coarse chromatin masses blending imperceptibly with scant parachromatin that have been described by workers, using the Romanowsky stain in light microscope methods. The narrow cytoplasmic rim reveals few or no structural details at this magnification. This micrograph is included especially for comparison with the succeeding Figures 6 and 8 to 12.

The normal medium-sized lymphocyte of Figure 5 (× 4800) likewise possesses a relatively narrow cytoplasmic band about a round or oval nucleus. Within the nucleus, large, coarse, chromatin masses are elongated into broad strands which in the smaller cells of this group inter-anastomose and in the larger members of this series present greater and greater tendencies to separation. The increased nuclear size can be attributed, for the most part, to an increase in the lighter areas of parachromatin and/or nuclear juice, both of which afford less resistance to the passage of electrons. Because of their large size, the chromatin strands afford suitable landmarks for differentiating such normal cells from the less mature forms described below.

TABLE 1

REPRESENTATIVE DIFFERENTIAL LEUKOCYTE COUNTS[12] IN PER CENT (SEE TEXT) IN A CASE OF CHRONIC LYMPHOCYTIC LEUKEMIA (200 CELLS)

DATE	MONOCYTES	NEUTROPHIL BAND FORMS	SEGMENTED NEUTROPHILS	EOSINOPHILS	LYMPHOBLASTS[12]	PROLYMPHOCYTES[12]	LYMPHOCYTES[12]
March 14, 1946	0	0	1	0	0.5	11	87.5
February 26, 1947	0	0	0	0	0	8.5	91.5
July 20, 1948	1	0.5	0	0.5	0	12.5	85.5

Figure 6 is an electron micrograph (× 4800) of a small lymphocyte obtained from the peripheral blood of a colored male patient, aged 70, suffering from chronic lymphocytic leukemia, lymphocytic type. This patient lived two and one-half years after diagnosis at his initial period of hospitalization in March, 1946 and at least three years after the onset of his symptoms. He had splenomegaly and lymphadenopathy. His leukocyte count when first seen was 110,000 per cu. mm.; this rose to a peak of 200,000 and slowly fell terminally to 77,000 per cu. mm. Light microscopic studies of the peripheral blood revealed a differential count made in accordance with the first report of the Committee on Hematologic Nomenclature,[12] as indicated in Table 1. Representative differential studies are likewise recorded at intervals in the same table. When differential counts were made in accordance with the criteria of Ferrata[11] and Strumia[30] (see discussion below) the same slides revealed the studies recorded in Table 2. The patient received roentgen therapy and transfusions of whole blood at intervals during his illness. The micrographs of Figure 6 and Figures 8 through 12, which were all taken from the peripheral blood of this patient, were obtained two and one-half months before his death in September, 1948.

Figure 6 depicts a small lymphocyte of the same size as the small lymphocyte of Figure 4. The small cytoplasmic bands of the two cells are comparable.

The chromatin masses of the leukemic lymphocyte of Figure 6 are smaller and their outlines are much more distinct. They are accentuated by the more obvious parachromatin spaces. In addition the dark, wavy, double ridge noted within the center of the nucleus suggests irregular membranous folding. Several small, deeply osmiophilic spherules are superimposed upon the nuclear pattern. These round, dark structures measure from 400 to 500 millimicrons in diameter.

Figure 7 is interposed in this series for purposes of background-contrast comparison with the other micrographs of this report. Actually, it is an electron micrograph (× 5200) of one of the more bizarre forms assumed by the anoxic sickle cell in sickle cell anemia.[26] This cell had been washed four times in physiologic saline solution. The clarity of the sharply contrasting background of Figure 7 hardly exceeds that of the similarly contrasting backgrounds of the cells in Figures 1 to 6 and 8 through 12 which had not been subjected to washing of any type prior to fixation.

TABLE 2

REPRESENTATIVE DIFFERENTIAL LEUKOCYTE COUNTS IN PER CENT (SEE TEXT), ACCORDING TO STRUMIA'S[30] DIFFERENTIATING CHARACTERISTICS (200 CELLS). SAME SLIDES WERE USED AS IN TABLE 1

DATE	MONOCYTES	NEUTROPHIL BAND FORMS	SEGMENTED NEUTROPHILS	EOSINOPHILS	LYMPHOBLASTS	PROLYMPHOCYTES	LYMPHOCYTES
March 14, 1946	0.5	0	2	0.5	3.5	89	4.5
February 26, 1947	1	0	0	0	2	92	5
July 20, 1948	1	0	2.5	1	2	91.5	2

A small leukemic lymphocyte similar to that in Figure 6 and a small sector of an adjacent lymphocyte are shown in Figure 8 (× 4800). In the intact cell the distinct, seemingly broken-up, small chromatin masses and the extreme irregularity and folding of the nuclear membrane are of interest. Several small, dark spherules are discerned overlying the nuclear lobulations.

Figure 9 is an electron micrograph (× 4800) of another leukemic small lymphocyte. It possesses the least differentiated of the nuclei studied in this series. A large, dark central nucleolar mass can be seen. The remainder of the nucleoplasm contains scattered small, globular chromatin pieces. At one portion of

All figures reduced to $\frac{4}{5}$ of original size.

FIG. 7. Electron micrograph of a bizarre red blood corpuscle from the blood in sickle cell anemia. × 5200.

FIG. 8. Electron micrograph of a small lymphocyte from the blood in chronic lymphocytic leukemia. × 4800.

FIG. 9. Electron micrograph of an immature, small lymphocyte from the blood in chronic lymphocytic leukemia. × 4800.

FIG. 10. Electron micrograph of a small lymphocyte from the blood in chronic lymphocytic leukemia. × 4800.

FIG. 11. Electron micrograph of a small lymphocyte from the blood in a case of chronic lymphocytic leukemia. × 4800.

FIG. 12. Electron micrograph of same cell as shown in Fig. 11 taken several minutes later and at higher magnification. × 6800.



FIGS. 7-12

the nuclear membranous arc, light clefts are observed which may represent shrinkage of nuclear material away from the membrane on the basis of fixation, desiccation or electron bombardment. A peculiar cytoplasmic budding, with central light areas of lessened resistance to electron passage, is well portrayed in various stages of development. Such cytoplasmic budding has been occasionally observed in leukemic leukocytes.

In contrast, Figure 10 (× 4800) is a micrograph representative of the most differentiated of the leukemic lymphocytes in this series. Even in this nucleus the chromatin aggregates, although large, are roughly globular and show some attempt at separation.

In Figures 11 (× 4800) and 12 (× 6800) which are electron micrographs of another small leukemic lymphocyte taken at different magnifications and at different time intervals, the nuclear pattern is again intermediate between that of Figures 9 and 10 and much more leptochromatic than that of the nucleus of the small control lymphocyte in Figure 4. A deep nuclear indentation common even in normal lymphocytes[10, 19] can be seen. Overlying the nucleus and also distinguishable in the cytoplasmic rim near the indentation are numerous, discrete, dark, osmiophilic spherules which measure approximately 250 to 500 millimicrons in diameter, only the largest spherule exceeding this range (by 250 millimicrons).

DISCUSSION

We have presented a method for electron micrography of cells within the smears of appropriately treated peripheral blood samples. The principal problem involved diminution without complete sacrifice of the macromolecular constituents of the plasma environment which render ordinary smears unsuitable for electron microscopy because of their production of a diffuse scattering of electrons and resultant loss of contrast. Reference to our electron micrographs will indicate the sharp background-contrast that has been achieved. With the attainment of a simple solution to this problem innumerable new difficulties again confront the investigator. Many of these difficulties are carried over from the available studies of function and structural organization with the optical microscope. Many arise from the micrographs themselves or the factors peculiarly pertinent to their preparation.

For an example of the former we may refer to the small, by ordinary standards, osmiophilic spherules noted particularly in our Figures 6, 11 and 12. We observe them not only in the cell body which lies external to the nuclear membrane but just as well in the thin film of cell body below or above the nucleus. Jones,[15, 16] using the phase microscope, has demonstrated that the mitochondria of lymphoid cells, especially of developmental forms of the erythrocytic series, occur in similar locations. Of equal import was the latter author's demonstration with Romanowsky dyes of mitochondria in such locations which appear as negative images and, therefore, contribute to the formation of parachromatin within the nuclear area. In a similar way the osmiophilic spherules in our micrographs which lie in the thin layer of cytoplasm within the observed nuclear area

appear as positive structures and so contribute to the nuclear pattern although it is doubtful that they actually contribute to the nuclear structure.

As an example of the problems pertinent to electron micrograph preparation we may cite the possibility of appreciable variation in the characteristics of cell constituents due to effects of electron bombardment in vacuum. Both duration and intensity of bombardment are important considerations. Chromatin particle size and other cellular particle sizes will, of course, be affected by such variables. With reference to this point it should be observed that Figure 12 (× 6800) was obtained several minutes after Figure 11 (× 4800). Obviously then, we have presented only qualitative pattern differences in our micrographs. The reader is referred to the recent work of Watson[32] for an extensive discussion of the many conditions affecting measurements in electron micrographs.

Our micrographs of small, circulating leukemic lymphocytes are representative merely of a single case of chronic lymphocytic leukemia. Still, their uniform portrayal of a small lymphocytic nucleus consistently less pachychromatic in appearance than the same type lymphocytic nuclei observed in May-Grünwald-Giesma stained, dry-fixed preparations is of interest. Although most hematologists[13, 18, 20, 21, 28, 33] agree that occasionally chronic lymphocytic leukemia of the *large* lymphocytic or even lymphoblastic cell type occurs, they also agree that the lymphocytes are usually *small* in this disease and resemble normal small lymphocytes as to coarseness of nuclear pattern. Naegeli,[20] for example, depicts these cells in color (his Fig. 93) and speaks of them as "altkernige Kleinezellen". Almost all authors[13, 18, 20, 21, 28, 33, 34] qualify their description of such small lymphocytes with differentiating characteristics of increased fragility or paucity in azurophil granules or possession of even less chromatin-parachromatin distinction than normal, or possession of a cart-wheel arrangement of nuclear constituents. Only a few, Ferrata,[11] Strumia,[30] Richter[28] suggest that the small, coarsely nucleated lymphocytes of chronic lymphocytic leukemia are, for the most part, immature forms at the prolymphocyte level of development. Strumia[30] describes the prolymphocyte as being a small cell with only a narrow rim of deeply basophilic, agranular cytoplasm about a coarse, deeply stained, round nucleus containing large chromatin blocks separated by distinct, "clear fissure-like spaces" (his Figs. 8 and 9, Plate II). Such a description is at variance with the more commonly accepted description[12] of the prolymphocyte in which a chromatin pattern *finer* than that of the mature lymphocyte is an important differentiating feature. In any event the finer chromatin pattern depicted in our micrographs of the small leukemic lymphocytes adds to the list of differentiating characteristics qualifying lymphocytic normality in chronic lymphocytic leukemia.

Any form of microscopy dealing with the non-living cell, deals with artificial organization. If such artificial organization is found to be constant after standard preparation of the non-living cell, then the artifacts so produced serve as useful tools. The electron microscopist, for the present at least, must utilize such tools. All of the micrographs in this paper, except that of the sickle cell, were of air-dried cells fixed in osmic acid. The simplicity of such a method for

a preliminary procedure is advantageous. There is no doubt that the nuclear pattern obtained after such treatment, although reproducible, is less desirable than the pattern obtained by the more complex methods reported earlier.[21] In this regard, as mentioned above, it is possible to utilize the author's modification of the Altmann-Gersh technic of freezing and dehydration in place of the steps of air-drying and osmic acid fixation. There is no doubt that the method of peripheral blood preparation for electron microscopy will lend itself to improvement, especially in the selection of better fixatives. Claude and Potter[8] have suggested that intranuclear chromatin filaments are destroyed when the cell is submitted to the usual means of fixation and resultant chromatin rearrangement gives rise to an alveolar appearance or concentration of chromatin into blocks. In preparations of lymphocytic leukemic spleen and smears of peripheral blood stained by the Feulgen technic (their Figs. 1 and 2), they demonstrated that the basophilic framework of the leukemic nuclei was composed of chromatin filaments similar in morphology to isolated chromatin filaments which they were able to obtain by mechanical separation (their Fig. 7). Sabin,[29] however, saw massive blotches of chromatin in the lymphocyte nucleus in the living state. In our own Figure 5 the chromatin arrangement within the lymphocyte nucleus suggests filamentous structures. The width of such strands varies from 0.5 to 1.2 microns, a range similar to that reported by Claude and Potter[8] for their isolated chromatin threads from leukemic lymphocytes.

The osmiophilic spherules of the cell body in the micrographs of leukemic lymphocytes are of additional interest. In view of Jones'[16] work on the appearance of mitochrondria as negative images in basophilic cell substance, dry-fixed preparations of leukemic lymphocytes were examined critically with the light microscope and many of the cells were found to contain numerous, small, unstained light spherules imbedded in the basophilic cytoplasm or actually overlying the nucleus. Tompkins and Cunningham[31] using the supravital method have commented on the abnormally abundant, fine, clumped mitochondria to be found in the lymphocytes of chronic lymphocytic leukemia. The possible occurrence of Golgi elements among the osmiophilic spherules cannot be excluded by micrograph studies alone. Cowdry[9] was unable to demonstrate staining of Golgi elements in lymphocytes after 15 days' immersion in 2 per cent osmic acid. In keeping with the observations of Tompkins and Cunningham,[31] we found osmiophilic spherules to measure from 0.25 to 0.5 microns in diameter and to be smaller than the diameters of mitochondria isolated from rat lymphosarcoma cells by Claude and Fullam[6] which varied from 0.6 to 1.3 microns.

SUMMARY

A method for the electron microscopy of cells within smears of peripheral blood samples has been outlined. After concentration of the desired cell layer, smears of the material were made upon formvar-covered glass slides. The smears were air-dried, immersed in 2 per cent osmic acid solution and washed in distilled water. The films were then transferred to screens by a modified stripping technic by which intact cells could be studied, not in replica, but directly. The electron

micrographs which were obtained exhibited sharp background contrast. Normal thrombocytes, neutrophilic leukocytes and small and medium sized lymphocytes were studied. The latter were contrasted with micrographs of small lymphocytes from a patient with chronic lymphocytic leukemia. Under the light microscope the small leukemic lymphocytes presented the customary coarse chromatin pattern of their nuclei. In contrast to both the light microscope pattern and the electron micrographs of normal lymphocytes, micrographs of the small leukemic lymphocytes depicted a finer chromatin pattern. The method and results have been discussed in relation to recent advances in the literature of leukocytic organelles.

REFERENCES

1. Barnes, R. B., Burton, C. J., and Scott, R. G.: Electron microscopical replica techniques for the study of organic surfaces. J. Appl. Physics, **16:** 730–739, 1945.
2. Bessis, M.: Ultrastructure du protoplasma des thrombocytes au microscope électronique. Compt. rend. Soc. de biol., **142:** 647–649, 1948.
3. Bessis, M.: International Society of Hematology, Exhibit, Buffalo, N.Y., 1948.
4. Bessis, M., and Burstein, M.: Études sur les thrombocytes au microscope électronique. Rev. d'Hemat., **3:** 48–68, 1948.
5. Bessis, M., and Burstein, M.: Une technique pour examiner les plaquettes au microscope électronique. Compt. rend. Soc. de biol., **142:** 27–28, 1948.
6. Claude, A., and Fullam, E. F.: An electron microscope study of isolated mitochondria. J. Exper. Med., **81:** 51–62, 1945.
7. Claude, A., Porter, K. R., and Pickels, E. G.: Electron microscope study of chicken tumor cells. Cancer Research, **7:** 421–430, 1947.
8. Claude, A., and Potter, J. S.: Isolation of chromatin threads from the resting nucleus of leukemic cells. J. Exper. Med., **77:** 345–354, 1943.
9. Cowdry, E. V.: The reticular material of developing blood cells. J. Exper. Med., **33:** 1–12, 1921.
10. Cunningham, R. S., and Tompkins, E. H.: The supravital method of studying blood cells. In: Downey, H., Handbook of Hematology. Vol. I. New York: Paul B. Hoeber, pp. 553–584, 1938.
11. Ferrata, A.: Le Emopatie. Vol. I. Milan: Soc. Edit. Lib., 1918. Cited by Richter.[28]
12. First report of the Committee for the Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs. Am. J. Clin. Path., **18:** 443–450, 1948.
13. Fowler, W. M.: Hematology. New York: Paul B. Hoeber, 1945.
14. Hovanitz, W.: An electron microscope study of isolated chromosomes. Genetics, **32:** 500–504, 1947.
15. Jones, O. P.: Mitochondrial influence on nuclear pattern of lymphoid cells. Anat. Rec., **100:** 681, 1948.
16. Jones, O. P.: Nuclear structure versus nuclear pattern. Blood, **3:** 967–986, 1948.
17. Jones, W. M.: Summarized proceedings of conference on the electron microscope. J. Scient. Instru., **24:** 113–119, 1947.
18. Kracke, R. R.: Diseases of the Blood and Atlas of Hematology. Ed. 2. Philadelphia: J. B. Lippincott Company, 1941.
19. Maximow, A.: The lymphocytes and plasma cells. In: Cowdry, E. V., Special Cytology. Vol. 1. New York: Paul B. Hoeber, pp. 319–367, 1928.
20. Naegeli, L.: Blutkrankheiten und Blutdiagnostic. Ed. 5. Berlin: J. Springer, 1931.
21. Piney, A.: Recent Advances in Haematology. Ed. 4. Philadelphia: The Blakiston Company, 1939.
22. Porter, K. R., and Thompson, H. P.: Some morphological features of cultured rat sarcoma cells as revealed by the electron microscope. Cancer Research, **7:** 431–438, 1947.
23. Rebuck, J. W., and Woods, H. L.: Electron microscope studies of blood cells in the hematopoietic organs and inflammatory exudates of man. Federation Proc., **6:** 398, 1947.
24. Rebuck, J. W., and Woods, H. L.: Electron microscope studies of blood cells. Blood, **3:** 175–191, 1948.
25. Rebuck, J. W., and Woods, H. L.: International Society of Hematology. Exhibit, Buffalo, N.Y., 1948.
26. Rebuck, J. W., Woods, H. L., and Monaghan, E. A.: Electron microscopy of sickle cells. Proc. Soc. Exper. Biol. and Med., **68:** 220–222, 1948.

27. Reed, C. I., and Reed, B. P.: Electron microscopy of erythrocytes from dog blood. Federation Proc., **6**: 185–186, 1947.
28. Richter, M. N.: Leucemia. In: Downey, H., Handbook of Hematology. Vol. 4. New York: Paul B. Hoeber, pp. 2882–3035, 1938.
29. Sabin, F. R.: Studies of living human blood cells. Bull. Johns Hopkins Hosp., **34**: 277–288, 1923.
30. Strumia, M. M.: Hematological Tables with Brief Notes. Bryn Mawr Hospital, Bryn Mawr, Pa. 1937.
31. Tompkins, E. H., and Cunningham, R. S.: The application of the supravital method to the study of the blood in pathological conditions. In: Downey, H., Handbook of Hematology. Vol. 1. New York: Paul B. Hoeber, pp. 585–640, 1938.
32. Watson, J. H. L.: Particle size determination with electron microscopes. Analytical Chem., **20**: 576–584, 1948.
33. Whitby, L. E. H., and Britton, C. J. C.: Disorders of the Blood. Ed. 5. Philadelphia: The Blakiston Company, 1946.
34. Wintrobe, M. M.: Clinical Hematology. Ed. 2. Philadelphia: Lea and Febiger, 1946.
35. Wolpers, C.: Zur Feinstruktur der Erythrocytenmembran. Naturwiss., **29**: 416–420, 1941.
36. Wolpers, C., and Ruska, H.: Strukturenuntersuchungen zur Blutgerinnung. Klin. Wchnschr., **18**: 1077–1081, 1111–1117, 1939.

THE VALUE OF BERYLLIUM DETERMINATIONS IN THE DIAGNOSIS OF BERYLLIOSIS*

F. R. DUTRA, M.D., J. CHOLAK, CH.E., AND D. M. HUBBARD, CH.E.

From the Kettering Laboratory of Applied Physiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio

Since the recognition of acute and chronic pulmonary berylliosis,[4,5,6] it has been found that the clinical diagnosis of this disease, especially the chronic form, may sometimes be quite difficult. In all cases of acute berylliosis a history has been obtained of occupational exposure to beryllium preceding the onset of symptoms. Some of the persons who developed chronic disease were also working in the beryllium industry at the time of the onset of symptoms, but in other instances exposure to beryllium had ceased as long as four years before symptoms appeared. We have encountered non-occupational chronic berylliosis in at least 9 persons who lived in the neighborhood of beryllium plants or handled the clothing of those working in beryllium plants.

It was believed that in some obscure illnesses in which berylliosis was suspected, examination for beryllium in fluids or tissues of the patients might aid in establishing or excluding this diagnosis. Accordingly, attempts were made to secure 24-hour samples of urine from patients who were ill with acute or chronic berylliosis and, as a control, from healthy persons currently working in beryllium plants. Analyses were also made of the urine of persons who had recovered from acute pulmonary berylliosis, some of whom were again working in the industry.

Analyses of sputum and blood samples were made in a few instances and of renal calculi from 4 persons with chronic berylliosis. In some fatal cases in which the subjects came to autopsy, tissues were obtained for analysis. In addition, tissues were analyzed from 11 persons who had had no known exposure to beryllium, including 7 who had died of Boeck's sarcoid with pulmonary involvement.

An accurate spectrographic method for the determination of minute quantities of beryllium in organic material[1,2] was employed. Some of the results were obtained in our earliest application of the analytic method, when a number of sources of contamination by traces of beryllium were not recognized or suspected. With increasing experience with the method,[2] it was possible to eliminate or minimize these contaminations, so that re-examination in questionable cases definitely established the presence or absence of beryllium. Such a re-examination was not possible in every case and a few of the extremely low positive findings may in fact represent contamination. It is believed that the positive results obtained in two† of eleven tissues from persons who had had no known exposure to beryl-

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 14, 1948.

† In one case, a value was obtained of 0.06 micrograms and in another case, 0.03 micrograms, per 100 grams of tissue.

Received for publication, October 11, 1948.

lium are instances of this type, since these analyses were made in the early stages of our work.

URINALYSIS

The data obtained by the analysis of 24-hour specimens of the urine of 27 persons are summarized in Tables 1 and 2 and Figure 1.

Table 1 suggests that most persons suffering from acute beryllium pneumonitis may be expected to have small quantities of beryllium in the urine. This

TABLE 1

BERYLLIUM IN URINE, OCCUPATIONAL PULMONARY BERYLLIOSIS
(Micrograms per Liter)

ACUTE (6 PATIENTS)	CHRONIC (14 PATIENTS)
0	0.32
0.3	1.0
0.3	1.7
0.5	0, 0.14**
1.2	0, 0.36**
0.9, 1.0, 0.5*	Negative in 9 others

* Specimens taken at intervals of 3 days.
** Specimens taken at intervals of 4 months.

TABLE 2

BERYLLIUM IN URINE, CHRONIC PULMONARY BERYLLIOSIS IN FIVE NON-OCCUPATIONAL CASES

PATIENT	MICROGRAMS PER LITER		PROBABLE SOURCE OF BERYLLIUM
A	0		Neighborhood of plant
B	0	Before BAL	Neighborhood of plant
	0	After 4 days of BAL	
	1.3	After completing 9 day course of BAL	
	0	3 months after course of BAL	
C	0.13		Neighborhood of plant
D	0		Husband's dusty clothing
E	0.12		Husband's dusty clothing

indication is supported by the data presented in Figure 1, which shows the daily urinary excretion of beryllium during acute pneumonitis in 2 patients who were treated by intramuscular injections of BAL (2,3-dimercaptopropanol). There were marked variations in the amounts of beryllium excreted from day to day, and in one case the 24-hour urine specimens taken at the end of the fifth and seventh days of BAL therapy contained no detectable beryllium. A single 24-hour specimen which is free from beryllium may, therefore, be insufficient to exclude a diagnosis of acute berylliosis. Incidentally, it may also be mentioned that the administration of BAL apparently did not affect either the course of the pneumonitis or the rate of excretion of beryllium in these 2 patients.

Table 1 also indicates that beryllium was found in at least one 24-hour specimen of the urine of 5 of 14 patients with occupational chronic pulmonary beryllosis, but single 24-hour specimens from the other 9 patients contained no beryllium. It should be noted that 2 of the persons who had positive specimens on one occasion had no detectable beryllium in their urine during another 24-hour period.

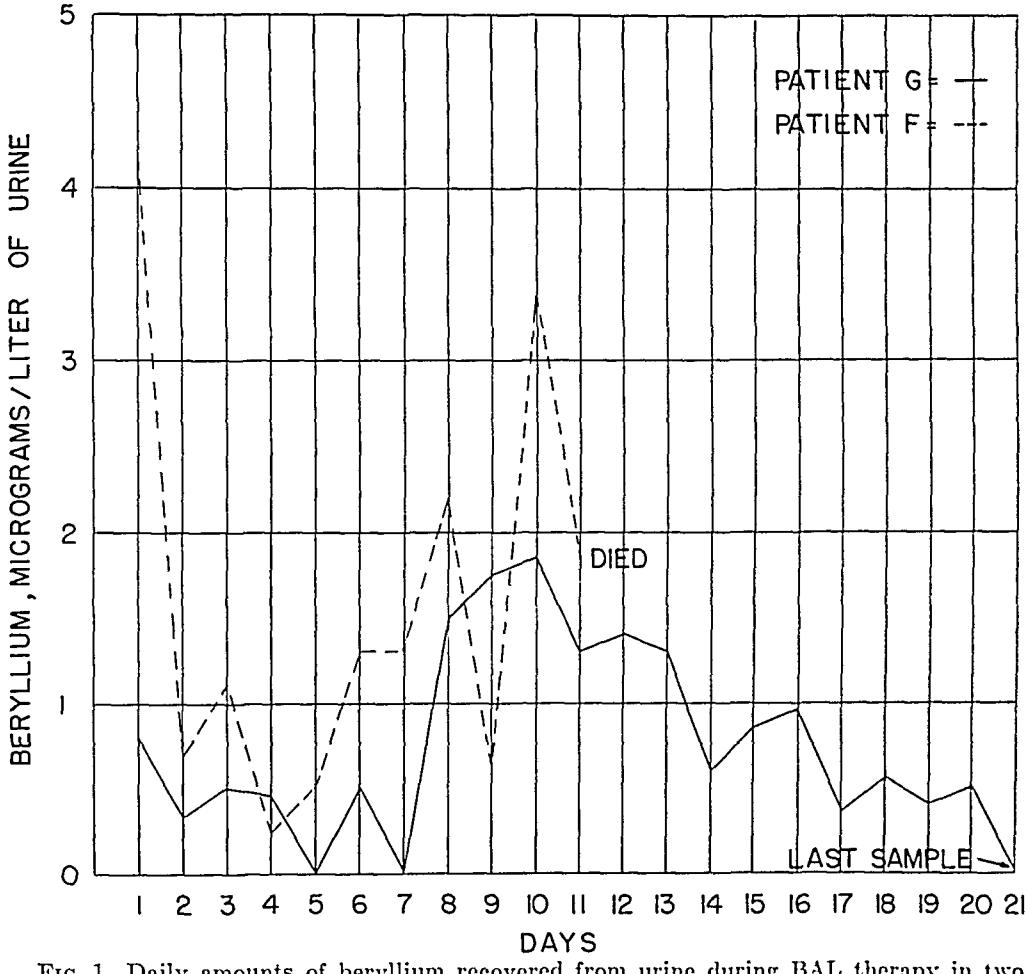


FIG. 1. Daily amounts of beryllium recovered from urine during BAL therapy in two patients with acute beryllium pneumonitis.

Table 2 lists the amount of excretion of beryllium in 24-hour specimens of the urine of 5 persons who had nonoccupational chronic pulmonary berylliosis. Beryllium was present in the 24-hour specimens of 3 of these subjects in detectable amounts on at least one occasion. In one instance, the beryllium was found in the urine after completion of a 9-day course of BAL.

Six persons who had recovered from acute pneumonitis and were no longer working in the beryllium industry were no longer excreting beryllium. Six persons who recovered from acute pneumonitis but who resumed work in the industry

were found to excrete the following amounts of beryllium in the urine, expressed in micrograms per liter: 0.25, 0.35, 0.40, 0.60, 0.75 and 1.0. Four other healthy persons while employed in the beryllium industry excreted the following amounts, in micrograms per liter of urine: 0, 0, 0.40 and 0.60.

ANALYSIS OF SPUTUM

In both acute and chronic pulmonary berylliosis the cough is nonproductive. Samples of the sputum of 3 persons suffering from acute pulmonary berylliosis and of 1 with chronic pneumonitis were examined but in no instance could beryllium be recovered.

ANALYSIS OF BLOOD

Specimens of blood were obtained from 2 persons with acute pulmonary beryl-

TABLE 3

EXAMINATION FOR BERYLLIUM IN RENAL CALCULI FROM FOUR PATIENTS WITH CHRONIC PULMONARY BERYLLIOSIS

WEIGHT OF CALCULUS	BERYLLIUM RECOVERED
mg.	*micrograms*
38*	0
47*	0.450
203*	0.035
69	0
48	0
11	0.035

* These three calculi were obtained from the same patient, May 24, 1947, June 24, 1947, and February 4, 1948.

liosis. In 1 person, a small amount of beryllium was detected in the blood (0.40 micrograms per 100 ml.).

BERYLLIUM IN RENAL CALCULI

Since beryllium is capable of forming practically insoluble compounds with phosphate in a slightly alkaline medium,[1] and since it was known that several patients with chronic pulmonary berylliosis had renal calculi, attempts were made to secure these calculi for analysis. A total of 6 stones were obtained from 4 patients with chronic pulmonary berylliosis and 3 of the stones (from 2 patients) contained minute amounts of beryllium (Table 3). It seems probable that these minute quantities of beryllium found their way into the calculi through incidental deposition from the urine and that the excretion of beryllium was not a factor in initiating the development of the calculi.

BERYLLIUM IN TISSUES IN FATAL CASES OF BERYLLIOSIS

The results of analyses of lungs and certain other tissues from 7 persons who died of acute pulmonary berylliosis and from 10 persons who died of chronic pulmonary berylliosis are shown in Table 4. The duration of illness in these patients

with acute pneumonitis varied from 14 to 35 days and from one to five years in those with chronic berylliosis. No correlation was found between the duration of exposure or the duration of illness with the amounts of beryllium which could be recovered from the lungs in either the acute or chronic form of the disease.

Lung tissue from 11 persons who had had no known exposure to beryllium dusts was also analyzed and in 2 of these persons, minute amounts of beryllium were found, the larger quantity being 0.06 micrograms per 100 grams of tissue. As stated above, it is possible that contamination was responsible for these two positive results.

In the small series of cases reported here, the distribution of beryllium in other tissues of the body has had no apparent or characteristic pattern.

TABLE 4

BERYLLIUM IN TISSUES, FATAL PULMONARY BERYLLIOSIS
(Micrograms Beryllium per 100 Grams Tissue)

TISSUE	7 PATIENTS WITH ACUTE DISEASE							10 PATIENTS WITH CHRONIC DISEASE									
Lung	9	12	18	20	98	128	220	0*	0.24*	0.33	0.93*	1.6*	1.7	12	16.5	20	78
Hilar node		4							0.67	0.25	6.4					138	
Liver		0.5	0.41	4					0		1.5			8.4	2.0	0	
Spleen		0.4	0.48	0					0	0	0.17			4.3	0.43	0	
Kidney		6	0.74	0					0	0		0.54		27.2	0.18	0	
Rib		0.3		0										13.5	0.44	3.0	
Heart		0.2	0.16													0	
Brain																0	
Thyroid																0	
Blood		0.4															

* Non-occupational disease.

DISCUSSION

It is apparent from the data presented, that persons who breathe in an atmosphere having beryllium-containing dust may be found to be excreting detectable amounts of beryllium in the urine. The amounts of beryllium in the urine of apparently healthy persons exposed to beryllium dusts are likely to be approximately of the same magnitude as in the urine of persons who are suffering from acute or chronic pulmonary berylliosis. Thus the presence of beryllium in the the urine is an indication not of berylliosis but of the absorption of beryllium. It appears that the chief clinical value of examining urine for beryllium lies in its indication of exposure to beryllium. If beryllium is found in the urine of a patient with pneumonitis, the possibility that the lung disease is caused by beryllium must be seriously considered.

The analysis of the lungs of persons believed to have died of berylliosis may aid in substantiating the diagnosis. With a single exception, in every case of proved berylliosis thus far subjected to analytic examination, measurable amounts of beryllium have been found in the lungs. In the one case in which beryllium was not recovered from the lungs, the amount originally present was

probably extremely small, since the exposure was not occupational but depended on residence in the neighborhood of a plant. Furthermore, the tissues had been preserved in formalin for 17 months before analysis, which may possibly have been a factor in eliminating any beryllium originally present.

The amounts of beryllium recovered from the lungs of persons who had died of acute berylliosis were generally higher than those recovered from cases of chronic berylliosis. However, our series is small and it will be interesting to learn if this situation holds true when data from a large number of persons are available. It will also be of value to learn if persons exposed to beryllium dust without developing clinical disease, show beryllium in the tissues in amounts comparable to those listed in Table 4. Until such data are available, the only value of analysis of lung tissue is to indicate if the individual inhaled appreciable quantities of beryllium-containing dusts. It is believed that the histologic alterations of acute or chronic pulmonary berylliosis are sufficiently characteristic[3] so that a positive diagnosis of berylliosis can be made by microscopic examination; this may be supported by tissue analyses for beryllium.

SUMMARY

1. The diagnosis of acute or chronic pulmonary berylliosis may, in some cases, be supported by analysis of urine for beryllium.

2. Beryllium has been isolated from the urine of persons working in beryllium plants who have no symptoms of berylliosis. Some of these persons have had acute pulmonary berylliosis previously.

3. The presence of beryllium in the urine of some persons with nonoccupational chronic berylliosis has aided in the diagnosis of the disease.

4. The excretion of beryllium in the urine of persons suffering with either acute or chronic berylliosis, varies from day to day, so that a few negative analytic results cannot be accepted as excluding berylliosis. It is possible that some patients with berylliosis do not excrete detectable amounts of beryllium at any time.

5. Appreciable quantities of beryllium have been recovered from the lungs of all patients who died from acute or chronic pulmonary berylliosis, except for one person with nonoccupational chronic berylliosis. Beryllium has also been recovered from other tissues in some of these patients.

REFERENCES

1. Cholak, J., and Hubbard, D. M.: Spectrographic determination of beryllium in biological material and in air. Analytical Chem., 20: 73–76, 1948.
2. Cholak, J., and Hubbard, D. M.: The spectrochemical determination of beryllium; increased sensitivity of detection in the cathode layer Analytical Chem., 20: 970–972, 1948.
3. Dutra, F. R.: The pneumonitis and the chronic granulomatosis peculiar to beryllium workers. Am. J. Path., 24: 1137–1166, 1948.
4. Hardy, H. L., and Tabershaw, I. R.: Delayed chemical pneumonitis occurring in workers exposed to beryllium compounds. J. Indust. Hyg. and Toxicol., 28: 197–211, 1946.
5. Martland, H. S., Brodkin, H. A., and Martland, H. S., Jr.: Occupational beryllium poisoning in New Jersey. J. Med. Soc. New Jersey, 45: 5–15, 1948.
6. VanOrdstrand, H. S., Hughes, R., DeNardi, J. M., and Carmody, M. G.: Beryllium poisoning. J.A.M.A., 129: 1084–1090, 1945.

NEEDLE ASPIRATION IN THE DIAGNOSIS OF LUNG CARCINOMA

Report of Experience with Seventy-Five Aspirations*

EMERSON Y. GLEDHILL, M.D., JOHN B. SPRIGGS, M.D., and CHAPMAN H. BINFORD, M.D.

From the Tumor and the Pathology Services, U. S. Marine Hospital, Baltimore, Maryland

During the period 1940 to 1948, needle aspiration of tissue from lung lesions has been used in the Tumor Clinic of this hospital as a diagnostic procedure in selected cases with suspected carcinoma of the lung. We believe that a report of our experience may be of interest.

According to Craver and Binkley,[1] the first reported diagnosis of carcinoma of the lung by microscopic examination of tissue obtained by aspiration was made by Menetrier in 1886. Since then a number of articles on the procedure have appeared in the European literature. In 1930, Martin and Ellis[4] at Memorial Hospital in New York City, reported their findings on biopsy of tissue from 65 aspirations, two of which were from the lung. Sharp[10] reported an additional case and described the technic in greater detail. Stewart[11] discussed "aspiration biopsy" in general from the histopathologic standpoint and interpreted his findings in 41 aspirations from suspected lung tumors. The method was further discussed by Martin and Ellis[5] and Martin and Stewart.[6] Lanari and Pavlovsky[3] in 1937 reported that in aspiration of tissue from 24 patients suspected of having carcinoma of the lung, a positive diagnosis was made on biopsy in 13 of the patients. Craver and Binkley[1] reported that in 92 lung aspirations a positive diagnosis was made in 60.8 per cent. They described the indications and operative technic in detail.

Between March 1, 1940 and July 1, 1948 the histologic diagnosis of carcinoma of the lung has been made in this hospital in 145 cases. Aspiration has been performed 75 times on 56 of the 145 patients in an effort to obtain material for diagnosis. Aspiration of tissue from the lung has been employed in our Clinic in patients with the following types of lung disease:

1. Patients in whom bronchoscopy and other technics fail or are inadvisable,
2. Those who are obviously inoperable, in whom accurate diagnosis is of great importance, and
3. Some patients with peripherally placed lung tumors or tumors that are inaccessible to the bronchoscope.

TECHNIC

1. *Equipment.* Specimen bottle with 10 per cent formalin, a skin germicidal agent, and the following sterile material: gloves, gauze sponges, 4 glass slides, two small (1 cm.) squares of blotting paper, 5 cc. syringe with #25 and #22 gauge needles for local anaes-

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, October 18, 1948.

thetic, 1 per cent procaine, sharp pointed scalpel (Bard-Parker #11 blade), one 20 cc. Record syringe, and an 18 gauge 10 cm. spinal needle with stylet.

2. *Roentgenographic localization.* Most lesions can be localized by routine postero-anterior and lateral chest roentgenograms. Stereoscopic films and fluoroscopic control were used in a few of the earlier cases, but, recently, the procedure has been performed more simply by not using these aids. Measurements of the distance to the central portion of the lesion from the central point of the sternum or from the dorsal spines are made laterally in the proper plane and the depth measured either from the anterior or posterior chest wall, whichever is closer. After these calculations have been made the skin is marked at a point over the lesion. If the aspiration is to be made laterally or posteriorly the patient is seated on a low stool with his elbows resting on a table. If an anterior approach is to be used he is placed supine on the table.

3. *Procedure.* The patient receives 10 mg. of morphine sulfate one-half hour prior to the aspiration. The skin is prepared as for any surgical procedure. A skin wheal is formed under the point marked and the chest wall is infiltrated to the pleura with 1 per cent procaine. A small puncture is made through the skin with a sharp pointed scalpel to prevent surface epithelium contamination of the aspirated material. The needle, with stylet in place, is inserted in accord with the calculations until, by a sense of resistance, it is felt to enter the tumor. The stylet is then removed and the Record syringe attached. Suction is obtained by withdrawing the plunger to the 20 cc. mark and maintained while the needle

TABLE 1

INITIAL DIAGNOSIS IN 145 CASES OF CARCINOMA OF LUNG

METHOD	CASES	PER CENT
Biopsy of aspirated tissue	44	30.3
Biopsy of tissue from bronchoscopy	36	24.7
Biopsy of tissue from metastasis	36	24.7
Thoracotomy	10	6.8
Sputum examination	3	2.5
Autopsy confirmation only	16	11.0

is advanced and withdrawn in the tumor area. The needle is advanced in slightly varying directions in the tumor area to obtain pieces of tissue. If because of puncture of a vessel or bronchus, blood or air enters the syringe, the needle is withdrawn slightly and suction re-applied. Before withdrawing the needle from the tumor and chest, suction should be released to avoid contamination of normal tissues and to prevent maceration and dispersement of the material by forceful impingement against the syringe walls or plunger. Pieces of tissue recovered from the syringe or expressed from the needle by the stylet are placed on the blotting paper and fixed in formalin. From the remaining material smears are prepared on the slides.

The smears are fixed with alcohol and stained with hematoxylin and eosin, while the clots on the blotting paper are processed as routine surgical material, embedded in paraffin and sectioned.

During this period several pathologists have participated in the diagnosis of our material. Questionable material has been referred to Dr. Fred W. Stewart of Memorial Hospital, New York City, for his opinion.

FINDINGS

Table 1 shows the initial method of diagnosis in this series. It is noted that in our experience the highest incidence of positive diagnosis was obtained on biopsy of aspirated tissue.

Table 2 indicates the comparative incidence of positive diagnosis on biopsy when tissue was aspirated and when tissue was obtained by bronchoscopy. Aspiration was performed 75 times in 56 patients and a positive diagnosis was rendered in 44 patients (78.5 per cent). Bronchoscopy was performed 102 times on 87 patients and a positive diagnosis was obtained in 36 patients (41.3 per cent).

Subsequent developments in the 44 patients having a positive diagnosis of aspirated tissue are shown in Table 3. Diagnoses were confirmed by autopsy, pneumonectomy or biopsy of tissue in 40 patients. The remaining 4 are living with clinical evidence of tumor.

TABLE 2

COMPARISON OF RESULTS OF BIOPSY OF TISSUE OBTAINED ON BRONCHOSCOPY AND OF TISSUE OBTAINED ON ASPIRATION

METHOD OF OBTAINING TISSUE FOR BIOPSY	NUMBER OF PROCEDURES	NUMBER OF PATIENTS	BIOPSIES POSITIVE	PER CENT POSITIVE
Bronchoscopy	102	87	36	41.3
Aspiration	75	56	44	78.5

TABLE 3

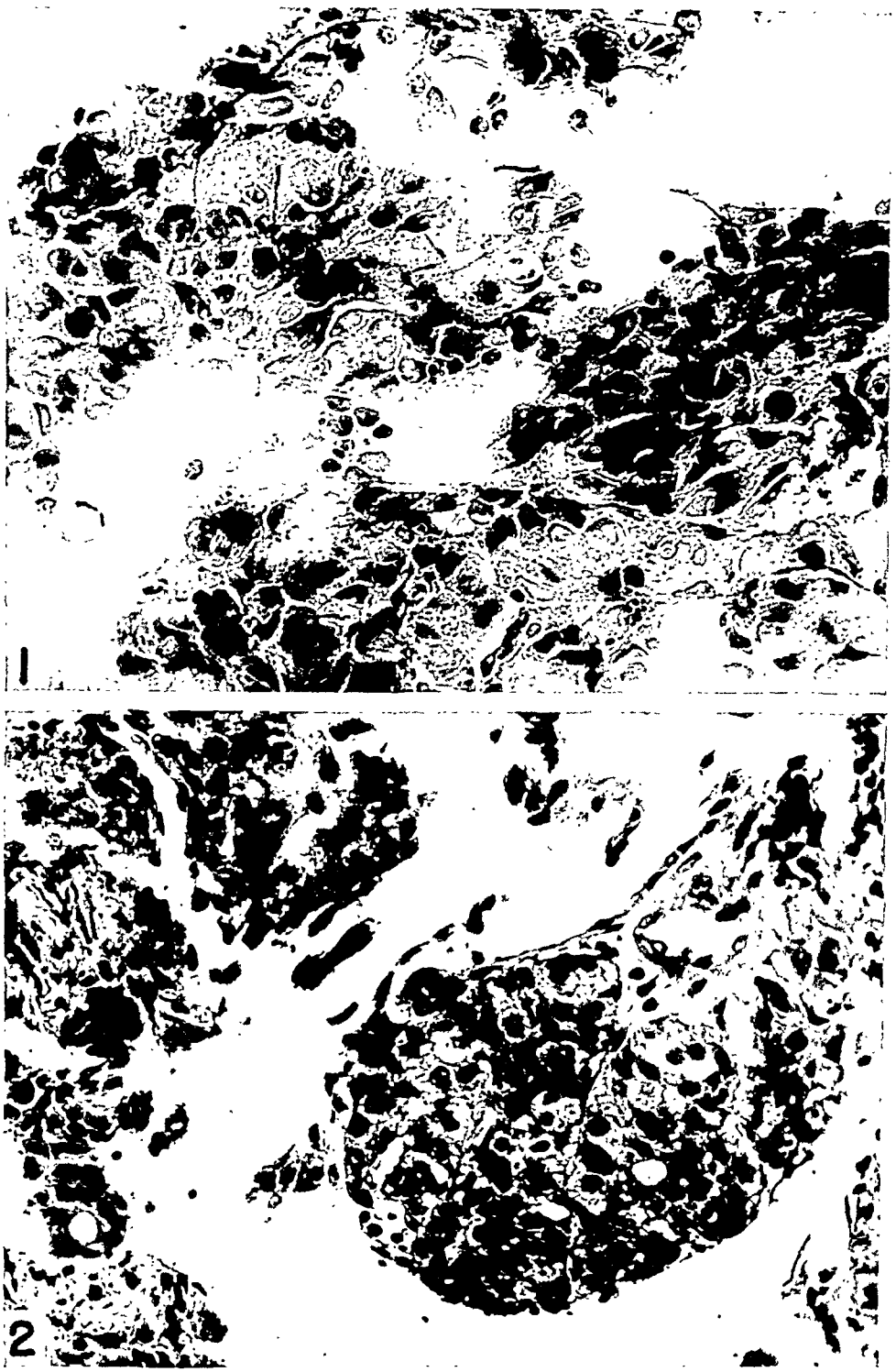
FOLLOW-UP CONFIRMATION OF DIAGNOSIS ON BIOPSY OF ASPIRATED TISSUE IN 44 PATIENTS

METHOD	NO. OF CASES
Autopsy	25
Exploratory thoracotomy	8
Pneumonectomy	3
Biopsy of metastatic tumor	4
Patients living with clinical evidence of tumor	4

DISCUSSION

Complications which could be attributed to the aspiration have been minimal in our series. In three patients a mild degree of pneumothorax resulted which required no special treatment. These probably would have gone unnoticed if postaspiration roentgenograms had not been made. We have observed 5 patients in whom slight transient hemoptysis occurred immediately following the procedure but we were unable to ascertain whether this was caused by the aspiration or was a part of the patient's symptom complex. Craver and Binkley[1] in 92 lung aspirations noted no undue complications. They noted occasional pain, but our patients did not complain of pain. Our patients likewise did not develop infections, pleural shock or mediastinal shifting.

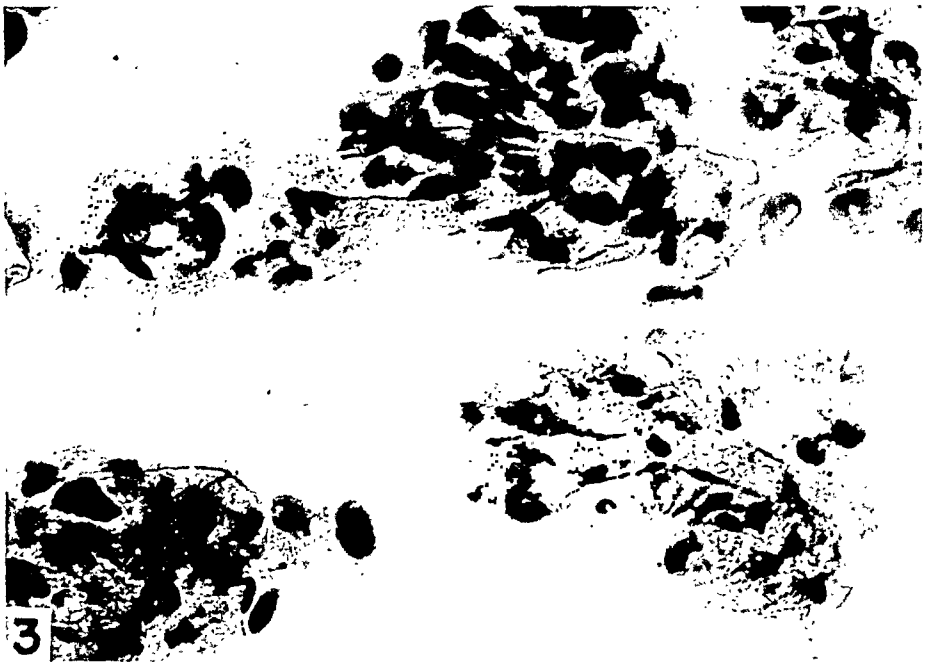
The criteria for the interpretation of biopsy of material aspirated from lung tumors does not differ from that used in biopsy of material obtained by aspiration from other parts of the body. The high degree of anaplasia frequently seen in lung carcinoma often aids the pathologist in making the diagnosis. It is our

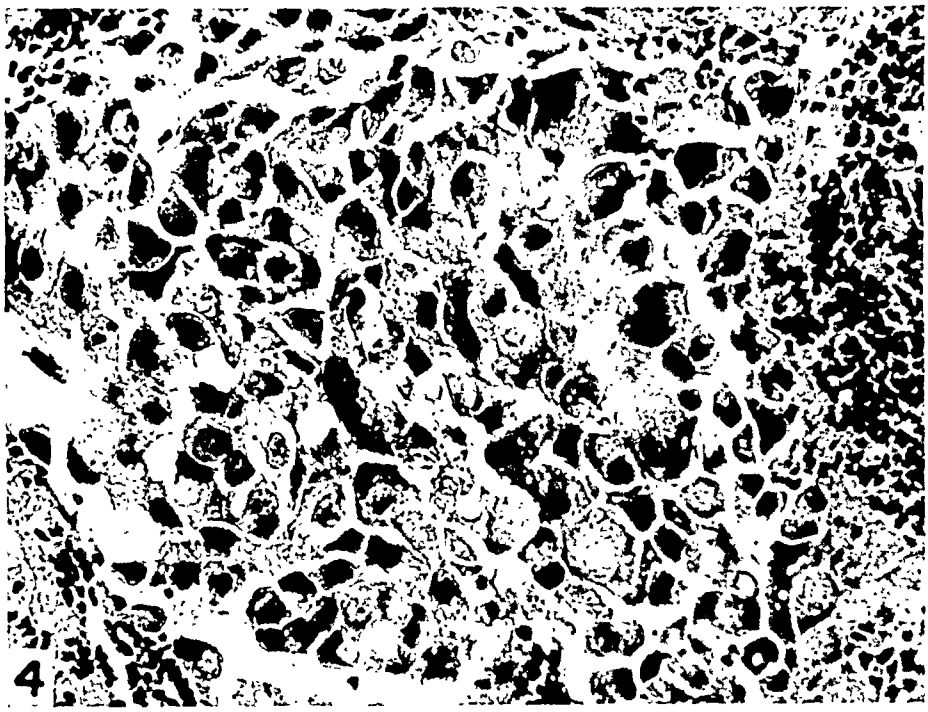


FIGS. 1 AND 2. Carcinomatous tissue aspirated by needle from left lung of man aged 76. Final diagnosis at autopsy, adenocarcinoma of bronchus.

FIG. 1. Smear of tissue. H. and E. × 400.

FIG. 2. Section of clot of tissue. Romanowsky stain. × 400.

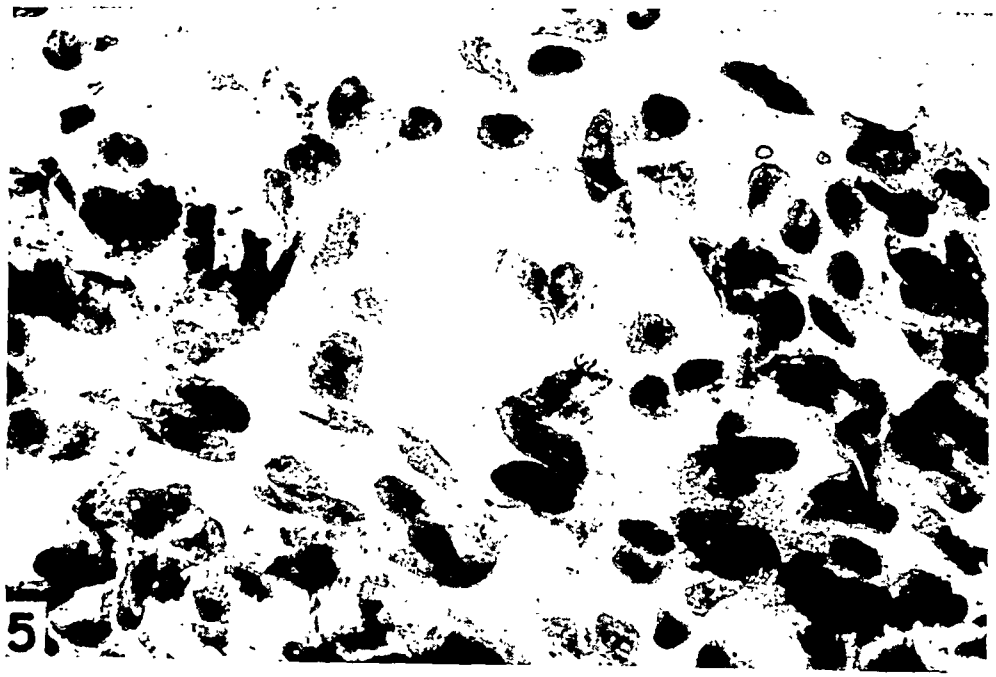


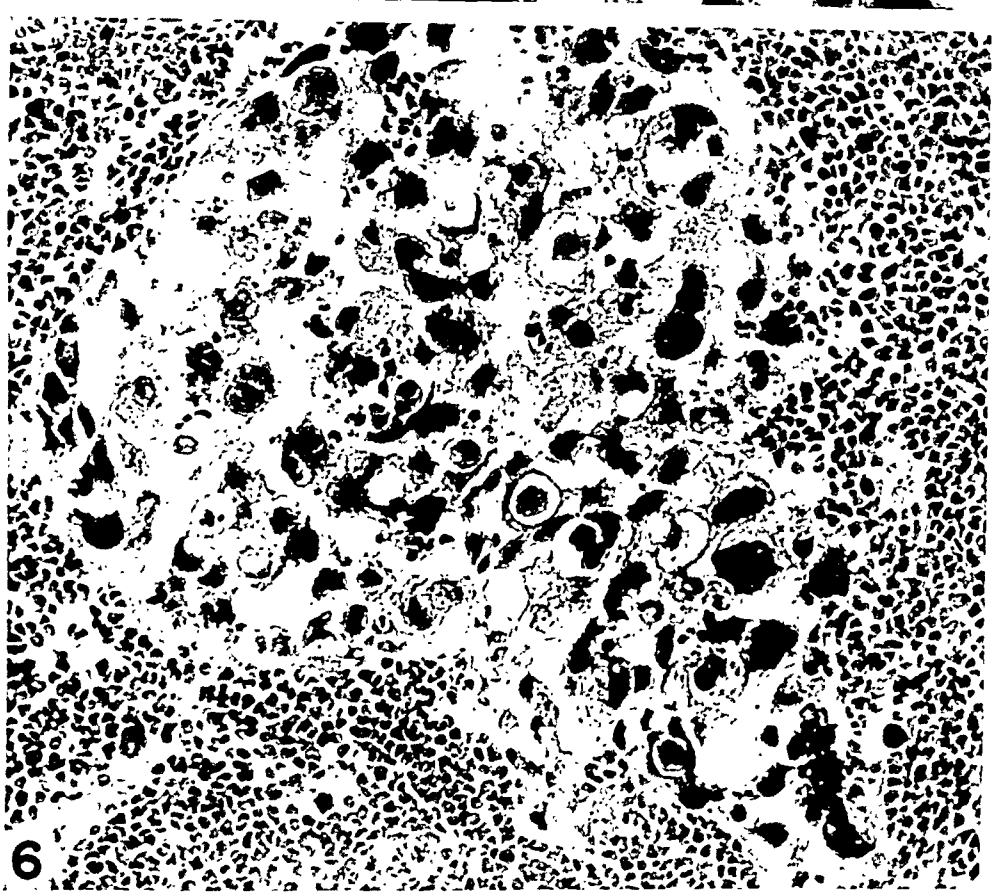


FIGS. 3 AND 4. Carcinomatous tissue aspirated by needle from right lung of man aged 56. Final diagnosis at autopsy, adenocarcinoma of bronchus.

FIG. 3. Smear of tissue. H. and E. × 400.

FIG. 4. Section of clot of tissue. H. and E. × 400.





FIGS. 5 AND 6. Carcinomatous tissue aspirated by needle from left lung of man aged 63. Final diagnosis at autopsy, anaplastic squamous cell carcinoma of bronchus.

FIG. 5. Smear of tissue. H. and E. × 400.

FIG. 6. Section of clot of tissue. Romanowsky stain. × 400.

impression that the information obtained from the sectioned clot is usually more helpful than that gained from smears. While in some instances, because of lack of material, the diagnosis is made on the smear or the clot alone, in the majority of cases the diagnosis represents interpretation of both. In most cases the pathologist can give only an opinion as to the presence or absence of evidence of cancer. In some cases, however, it may be possible to indicate the type of carcinoma and/or the degree of anaplasia exhibited. The confidence of the pathologist in this method of diagnosis will increase in proportion to his opportunity of seeing material obtained by aspiration of pulmonary or other lesions. Figures 1, 2, and 3 illustrate the histologic findings in 3 cases.

The advisability of aspirating tissue from tumors of the lung for biopsy has been the subject of some controversy. Ochsner and DeBakey[7] in 1939 stated that such aspiration was a useful procedure where bronchoscopy was unsuccessful and could be performed with relative safety and a fair degree of accuracy. However in 1942, they[8] condemned the procedure because implant-carcinoma occurred in the pleura of two patients following removal of the specimen. They gave no further details of these cases. They mentioned a similar case reported by Dolley and Jones[2], but a review of this report reveals that the pleural implant followed aspiration of pleural fluid containing cancer cells. Rienhoff[9] condemns aspiration of the lung as a diagnostic measure for biopsy but cites no personal experience with this method. No needle-tract implantation has been observed in any of our patients all of whom have been followed as shown in Table 3, but postmortem histologic study of needle tracts has not been performed. Craver and Binkley[1] in their series of 92 aspirations reported no implantations.

We have found aspiration with biopsy a valuable aid in diagnosis, and in following treatment of carcinoma of the lung. We do not, however, regard aspiration as a simple procedure because it is conceivable that a serious complication may occur.

SUMMARY

Aspiration of tissue from the lung for biopsy has been used in this clinic 75 times on 56 patients in a series of 145 patients with carcinoma of the lung without serious complications. By this method a positive diagnosis was obtained in 44 of the 56 patients (78.5 per cent). Bronchoscopy, performed 102 times on 87 patients, enabled a positive diagnosis on biopsy in 36 patients (41.3 per cent).

In our experience the method is safe and is a valuable aid in enabling the diagnosis of carcinoma of the lung to be made on biopsy.

Acknowledgment. For his aid in preparing this report, we wish to thank Dr. J. E. Wirth, Director, Tumor Clinic, U. S. Marine Hospital, Baltimore, who initiated and encouraged the use of aspiration for biopsy as a diagnostic procedure.

REFERENCES

1. Craver, L. F., and Binkley, J. S.: Aspiration biopsy of tumors of lung. J. Thoracic Surg., 8: 436–463, 1939.
2. Dolley, F. S., and Jones, J. C.: Lobectomy and pneumonectomy for lung suppuration and malignancy. Lancet, 69: 162–168, 268–278, 1939.

3. LANARI, A., AND PAVLOVSKY, A.: La puncion en el diagnostico del cancer del pulmon. Dia méd. Ano 9, No. 31, 1937.
4. MARTIN, H. E., AND ELLIS, E. B.: Biopsy by needle puncture and aspiration, Ann. Surg., **92:** 169–181, 1930.
5. MARTIN, H. E., AND ELLIS, E. B.: Aspiration biopsy. Surg., Gynec. and Obst., **59:** 578–589, 1934.
6. MARTIN, H. E., AND STEWART, F. W.: Advantages and limitations of aspiration biopsy, Am. J. Roentgenol., **35:** 245–247, 1936.
7. OCHSNER, A., AND DEBAKEY, M.: Primary pulmonary malignancy; treatment by total pneumonectomy. Surg., Gynec., and Obst., **68:** 435–451, 1939.
8. OCHSNER, A., AND DEBAKEY, M.: Significance of metastasis in primary carcinoma of lungs, J. Thoracic Surg., **11:** 357–387, 1942.
9. RIENHOFF, W. F., JR.: The present status of the surgical treatment of primary carcinoma of the lung. J. A. M. A., **126:** 1123–1128, 1944.
10. SHARP, G. S.: Diagnosis of primary carcinoma of lung by aspiration. Am. J. Cancer, **15:** 863–870, 1931.
11. STEWART, F. W.: Diagnosis of tumors by aspiration. Am. J. Path., Suppl., **9:** 801–812, 1933.

MALIGNANT PHEOCHROMOCYTOMA AND HYPERTENSION

A Case Report*

JEROME CHAMOVITZ, M.D., AND HERBERT FANGER, M.D.

From the Department of Pathology, Peter Bent Brigham Hospital, Boston, Massachusetts

Pheochromocytoma, chromaffin cell tumor of the adrenal medulla, is now a well recognized clinical and pathologic entity, 176 cases having been found in a recent survey of the world's literature.[11] In the past twenty years it has become recognized as a curable cause of paroxysmal and chronic hypertension, some 45 surgical cases having been reported by 1945.[25] Ninety per cent of the tumors reported have been benign. Yet, even the benign tumors may lead to a fatal outcome from hypertension unless there is surgical intervention. Contrary to the impression gathered from the older literature[7, 9, 20] that the malignant tumors were rarely associated with hypertension, a review of the recent literature leads to an opposite opinion.

The case herein reported represents a malignant pheochromocytoma with hypertension. It was previously the subject of a clinical case report in 1944 by Thorn *et al.*,† following surgical removal of the tumor with temporary clinical improvement. Recurrence of hypertension one year following operation led to eventual cardiac and renal failure and death. Details of the final clinical course and autopsy findings are summarized below.

CASE HISTORY

J. B., forty-two years old, the wife of a physician, was discovered to be hypertensive during a routine examination in 1937, nine years before her death. She continued to show an elevated blood pressure thereafter, though neither subsequent symptoms nor hypertension were ever paroxysmal in character. In April, 1943, an intravenous pyelogram indicated the possibility of a left adrenal tumor. At operation, a tumor of the left adrenal was removed and no gross evidence of lymph node invasion or local extension was found. However, microscopic examination of the tumor revealed extensive capsular invasion, some extension beyond the capsule and invasion of the blood vessels. Because of the possible malignancy, x-ray therapy was given over the region of the left kidney.

Following operation, the blood pressure remained at normal levels for one year, following which the pressure gradually returned to the previous high levels. Exploration of the right adrenal gland in October, 1944, failed to disclose a second tumor. Biopsy of tissue taken from the right kidney at that time revealed marked arteriolar nephrosclerosis. Persistent elevation of blood pressure continued and death occurred in February, 1946, from cardiac and renal failure.

AUTOPSY REPORT

At autopsy, the major diagnoses were: (1) recurrent malignant pheochromocytoma of left adrenal with local extension and with metastasis to mediastinal lymph nodes; (2) arteriolar nephrosclerosis, severe, (malignant nephrosclerosis); (3) left hydroureter and hydronephrosis, secondary to ureteral compression by tumor; (4) multiple renal arterial

* Received for publication, October 20, 1948.

† We are grateful to Dr. Thorn for permission to report this case.

thrombosis with infarction, left; (5) hypertensive heart disease, as evidenced by cardiomegaly and left ventricular myocardial hypertrophy; (6) cardiac failure, as evidenced by mural thrombosis of right auricle, bilateral hydrothorax, ascites, pulmonary congestion, and chronic passive congestion of liver and gastrointestinal tract; (7) pulmonary embolism with infarction of lower lobe of right lung.

In the region of the original adrenal tumor surgically removed three years previously, tumor tissue capped the upper pole of the left kidney. This tumor extended down along the medial border of the left kidney into a large mass (10 x 8.5 x 4 cm.) located between the two kidneys. The tumor was pink-gray, firm, and irregularly lobulated. Intimate fusion with infiltrated regional peri-aortic lymph nodes made it difficult to differentiate the original structural components of the mass. On cross section, the tissue was pink-gray, lobulated, and revealed scattered areas of necrosis and hemorrhage. The tumor compressed the aorta posteriorly and surrounded the left renal artery. The lumen of the latter had been narrowed to the diameter of a probe by this extrinsic pressure but the vessel was not thrombosed. Near its origin, the left ureter similarly was compressed by the tumor with resultant proximal hydroureter and hydropelvis.

The left kidney, bound down by dense adhesions, was shrunken, weighed 100 Gm., and its upper pole was distorted and compressed by the adjacent tumor. Separation of the moderately adherent capsule showed a granular surface and numerous petechiae. The cortex was reduced in thickness. As noted above, the renal pelvis and proximal ureter were dilated. The right kidney was of average size, weighed 150 Gm., and was remarkable only because of petechiae in the cortex and a fine granularity of the cortical surface.

A focus of metastatic tumor 4 x 2 x 1.5 cm., was found in the mediastinum, located between the trachea and esophagus and about 3 cm. proximal to the bifurcation of the trachea. On cross section it appeared to be composed of several lymph nodes bound together and infiltrated by pink-gray firm tissue.

Microscopically the tumor seen at autopsy resembled the original surgical specimen except for somewhat greater pleomorphism, more extensive invasive tendency, and increased fibrosis (Figs. 1 and 2).

Sections were stained with hematoxylin-eosin, Masson's trichrome, aniline blue and Schmorl's stains. The basic feature was irregular masses of cells surrounded by collagenous fibrous tissue of variable thickness. In many fields the tumor tissue appeared retracted from the surrounding fibrous stroma, which probably represented shrinkage rather than a characteristic histologic pattern. The fibrous tissue was quite dense in some sections of the tumor, and appeared to be growing at the expense of the remnants of tumor tissue. There were occasional foci of ischemic necrosis, and this as well as the fibrosis may possibly have been caused by the x-ray therapy, although the characteristic vascular changes resulting from irradiation were not found.

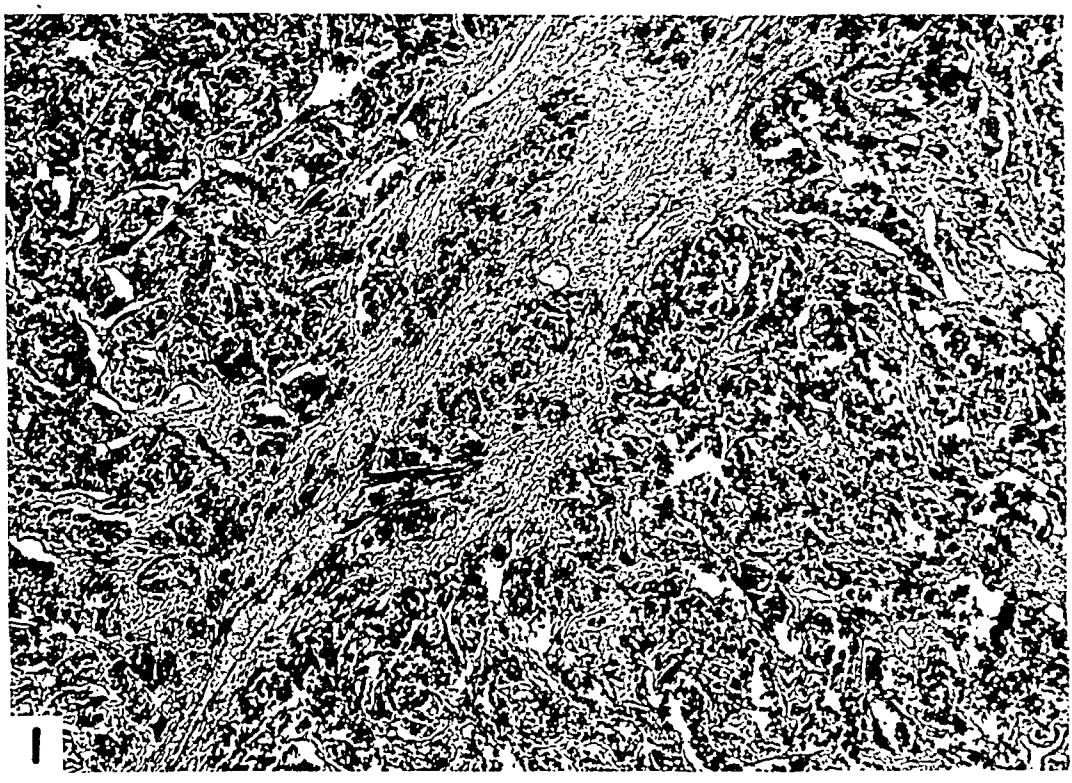
The tumor cells, although poorly demarcated, were characteristically polygonal in shape, and of large size, ranging from 25 to 35 microns in diameter. An occasional giant mononuclear cell measured from 80 to 100 microns in largest diameter, and consisted of an ill-defined mass of ragged acidophilic cytoplasm having a single gigantic hyperchromatic nucleus. On the whole, the nuclei of the cells were large, filled the cells well, and had distinct nuclear membranes. There were 1 to 3 nucleoli with an occasional nucleus containing a nucleolus of giant size which appeared to contain a large vacuole. The chromatin was for the most part well dispersed in the nuclei.

In the hematoxylin-eosin stained sections the cytoplasm, usually scanty, tended to be slightly acidophilic and finely granular. In the trichrome-stained sections, the granularity of the cytoplasm was more prominent and occasionally was brown in color, the so-called chromaffin reaction. Although there was considerable pleomorphism, only a few mitotic

FIG. 1. Survey of tumor pattern. Masson's trichrome stain. × 45.

FIG. 2. Pleomorphism of the tumor and granularity of cytoplasm. Masson's trichrome stain. × 765.

FIG. 3. Tumor cells in blood vessel. Hematoxylin-eosin stain. × 140.







FIGS. 1–3

figures were seen. Tumor cells extensively invaded blood vessels and lymphatics, as well as the fibrous capsule (Fig. 3).

Microscopic sections from the mediastinal mass showed lymph nodes almost completely replaced by tumor tissue similar to that in the primary growth. Sections of the original surgical specimen gave a chromaffin reaction as demonstrated by the Schmorl stain, characterized by an olive-green coloration in the cytoplasm of some of the cells. In the tissue fixed in Regaud's solution and stained with hematoxylin-eosin, there were found areas where the tumor cells contained yellowish green granules or vacuoles and there was a similar coloration in the intercellular fluid. The chromaffin reaction was weakly positive in the tumor tissue removed at autopsy, since the tissue was fixed in Zenker-acetic acid and the acid was believed to have denatured most of the cytoplasmic reducing substance which produces the chromaffin reaction.

Microscopically, the kidneys showed severe arterial disease, as evidenced by marked subendothelial fibroblastic proliferation of the arterioles, producing the so-called "onion-peel" appearance, and by occasional arteriolonecrosis. The larger vessels showed pronounced intimal proliferation. The glomeruli frequently were hyalinized and showed rare foci of necrosis. Small arteries occasionally were occluded by thrombi, with associated infarction.

DISCUSSION

In attempting to evaluate the clinical picture produced by malignant pheochromocytoma, one is struck by the paucity of accurate clinical and pathologic information available in the literature. Many earlier reports are sketchy in detail, so that accurate over-all evaluation is actually impossible. Specifically, it has been stated in the literature[9, 13, 14, 20, 29] that malignant pheochromocytomas, unlike the corresponding benign tumors, are neither physiologically active nor commonly associated with hypertension. In recent years several reports have appeared like the present one, in which malignant pheochromocytomas were unquestionably associated with hypertension. In an attempt to emphasize relationship, the evidence has been herein briefly reviewed (Tables 1 and 2).

The original impression regarding malignant pheochromocytomas and hypertension stemmed from one of the earlier reviews written in 1932 by Eisenberg and Wallerstein[9] in which it was not only stated that malignant pheochromocytomas were not associated with hypertension, but in addition, that all known malignant pheochromocytomas were bilateral. Of 53 cases then reported in the literature, they considered 5 to be definitely malignant, basing their judgment on the criterion of metastasis. Analysis of their data (Table 1, Part A), however, shows that in only 3 of the 5 cases were blood pressure figures available and bilateral involvement reported.

In 1937, Edward,[8] in reviewing the literature, found 55 cases of pheochromocytoma which were malignant; only one of these was bilateral. Only four tumors presented gross evidence of malignancy (metastasis) (Table 1, Part B).

In 1942, McGavack *et al.*[20] reviewed the data on 9 cases of malignant pheochromocytoma, which included the 5 cases originally reviewed by Eisenberg and Wallerstein. Recognizing the difficulty in differentiating benign from malignant pheochromocytoma on the basis of histologic appearance, they accepted only those cases which showed gross evidence of metastasis. Their data are summarized in Table 1, Part C. They concluded that paroxysmal hyperten-

sion is absent in malignant pheochromocytoma. None of the four cases added in their review showed bilateral tumors.

In a review of the literature in the English language from 1941 through June 30, 1947, we found 12 cases of pheochromocytoma in which some evidence of malignancy was found. This includes the current case which was originally

TABLE 1

CASES OF MALIGNANT PHEOCHROMOCYTOMA PREVIOUSLY REPORTED

AUTHOR	YEAR	GROSS EVIDENCE OF MALIGNANCY	INVOLVEMENT	BLOOD PRESSURE
A. QUOTED FROM EISENBERG AND WALLERSTEIN, 1932				
Masson	1923	Metastasis to lymph nodes and liver	Not reported	Not stated
Gravier and Bernheim	1924	Metastasis to mediastinum	Bilateral	Hypotensive
Bonnamour *et al.*	1927	Metastasis to lymph nodes and pleura	Not reported	Not stated
King	1931	Metastasis to liver, lungs, bones and brain	Bilateral	Normal
Lazarus and Eisenberg	1932	Metastasis to liver, lungs, bones, intestines, glands and gum	Bilateral	Normal
B. QUOTED FROM EDWARD, 1937				
Buchner	1934	Metastasis (site not stated)	Bilateral	Hypertensive, but figures not stated
Ernould and Pilard	1934	Widespread metastasis	Unilateral	Paroxysmal
McKenna and Hines	1935	Metastasis (site not stated)	Unilateral	160 to 190 mm. (systolic)
Kaulbach	1937	Metastasis to lungs	Unilateral	Hypertension
C. CASES ADDED BY MCGAVACK *ET AL.*, 1942				
Lewis-Geschickter Case #47632	1934	Metastasis to kidney and retroperitoneal lymph nodes	Unilateral	Not stated
Case #43558		Neighboring lymph nodes	Not stated	Not stated
McGavack *et al.*	1942	Metastasis to bones, lungs, liver, skin, etc.	Unilateral	Hypotensive

reported by Thorn *et al.*[27], in 1944. In attempting to differentiate malignant from benign pheochromocytomas, we also were struck by the difficulty in interpreting the microscopic findings. The difficulties of establishing a correct diagnosis were also aggravated by the meager details of gross and microscopic findings. Rather than omit any possible malignant tumors, we have tabulated the diagnoses as they have been reported by the respective authors. Gross metasta-

sis is incontrovertible evidence of malignancy, but we agree with the suggestion of Washington *et al.*[28] that evidence of local invasion be added to the criterion of McGavack *et al.* Of the 12 cases reviewed, eight showed gross evidence of malignancy, while four presented only a suggestive microscopic picture (Table 2).

It is interesting to note that in the 11 cases in which blood pressure was reported, all were associated with hypertension. Of these, 8 were paroxysmal in character, 2 presented the picture of malignant hypertension, and 1 (our own)

TABLE 2

CASES OF MALIGNANT PHEOCHROMOCYTOMA REPORTED FROM 1941 TO JUNE 30, 1947

AUTHOR	YEAR	EVIDENCE OF MALIGNANCY		INVOLVEMENT	TYPE OF HYPERTENSION
		Gross	Microscopic		
Evans and Stewart	1942	Not reported	Present	Unilateral	Paroxysmal
Kenyon	1944	Not reported	Present	Unilateral	Paroxysmal
Cahill, Case 1	1944	Metastasis to liver and lymph nodes	Not reported	Unilateral	Paroxysmal
Case 5	1944	Metastasis to meninges	Not reported	Unilateral	Not reported
MacKeith	1944	None	Present	Unilateral	Paroxysmal
Thorn *et al.*	1944	Metastasis to mediastinum	Present	Unilateral	Chronic
Espersen and Dahl-Iversen	1946	Local venous invasion	Present	Unilateral	Malignant
Soffer *et al.*, Case 1*	1946	Metastasis to liver	Present	Unilateral	Paroxysmal
Case 2	1946	Nearby metastatic tumor	Present	Unilateral	Paroxysmal
Washington *et al.*	1946	Invasion of inferior vena cava	Present	Unilateral	Malignant
Barlow	1946	Adherence of tumor to diaphragm, pancreas and stomach	Present	Unilateral	Paroxysmal
Mayock and Rose	1947	None	Present	Unilateral	Paroxysmal

* We are indebted to Dr. Louis Soffer, New York City, for this follow-up information, not available in the original article, where the tumor was originally presumed to be benign.

demonstrated the unique picture of chronic hypertension of nine years' duration without paroxysmal episodes. This is in striking contrast to McGavack's group, where 8 of 9 cases were unassociated with hypertension. In addition, all 12 of the cases reviewed showed unilateral involvement.

At present there is no adequate explanation for the presence of hypertension with some malignant pheochromocytomas and not in others. It is interesting to speculate that the presence or absence of hypertension may depend on the degree of differentiation of the tumor. The benign pheochromocytoma is a tumor of adult cells (pheochromocytes) of the adrenal medulla. These tumors

are said to have a high adrenalin content which histochemically is related to the so-called chromaffin reaction.[3, 18, 23] The precursor of the pheochromocyte is the pheochromoblast which, in contrast to the adult cell, does not stain with chromium salts.[2, 24] This suggests the absence of adrenalin formation by these cells and may indicate that the tumors of immature cells are nonfunctioning. In the case of the highly malignant undifferentiated tumor composed of immature pheochromoblasts only, one might possibly have an explanation for the absence of hypertension. Such tumors probably are very rare, because in the average example one would expect some functioning tumor cells which probably are increased in number over that in the normal adrenal medulla, thus causing excessive adrenalin formation and hypertension.

It is interesting to note that several cases[9, 17, 20] associated with normal blood pressure developed a rapidly progressive downhill course accompanied by widespread metastasis in contrast to those associated with hypertension which predominantly ran a slower course and showed a limited tendency to metastasize.

There are several factors to be considered in evaluating the etiology of hypertension in this case. These are, the adrenalin-secreting tumor, the x-ray treatment to the left kidney region, and the constriction of the left renal artery by tumor. It is our impression that the primary cause is the adrenalin-secreting tumor which caused marked vascular changes leading to irreversible hypertension. Biologic assay of the tumor removed in the original operation (1943) revealed a significant content of adrenalin. The finding of severe arteriolar sclerosis in the non-irradiated right kidney within a year of irradiation of the left made it seem unlikely that x-ray treatment contributed to the hypertension. At autopsy, the finding of constriction of the left renal artery by tumor suggests the possibility of a "Goldblatt" mechanism. There is marked vascular disease in both kidneys although somewhat more prominent on the left side. Although the constricted left renal artery may ultimately have contributed to the hypertension, the existence of hypertension at the time of the first operation and while the tumor was still confined to the upper pole of the kidney indicates that renal artery constriction was probably of minor and secondary importance. Therefore, the malignant pheochromocytoma is regarded as the basic cause of the hypertension.

There are two features in the histopathology of these tumors which are worthy of emphasis: (1) The significance of the so-called chromaffin reaction, and (2) the difficulty of differentiating benign and malignant pheochromocytomas by their microscopic appearance.

The chromaffin reaction refers to the brown coloration assumed by the cytoplasm of the cells of adrenal medulla when exposed to chromium salts. This reaction has been explained as being caused by the oxidation of various organic chemical compounds of the cytoplasm which have already formed adrenalin or related compounds and which, as the result of oxidation, have formed "brown polymers". This reaction is not exclusively the property of chromium salts but of various oxidizing substances such as the periodates which will also cause the coloration[3]. The amount of brown pigment coloration produced is related

to the amount of adrenalin-like substance produced in the cells. Such a reaction is markedly reduced if the exposure of the tissues to the chrome salts or other oxidizing agents is delayed for more than several hours. In the case of Zenker fluid used as a fixative it is important that the acetic acid be withheld since the acid apparently destroys the chromaffin reacting substance.

After reviewing the histology of seven pheochromocytomas*, it became apparent that cellular pleomorphism did not invariably indicate the presence of malignancy. Benign tumors may be vascular and are comprised of groups of polygonal cells closely approximated by thin-walled vascular spaces. Not only do the cells vary in size and shape, but they may be multinucleated. Even the benign tumors are so richly vascularized and poorly organized that displacement of cells into vascular spaces incident to the preparation and processing of the microscopic sections may create a false impression of malignant vascular invasion. It has, therefore, been suggested by some authors[20] that the diagnosis of malignancy be made only on the basis of evidence of metastasis of the tumor or of local invasion.

SUMMARY

A case of malignant pheochromocytoma with hypertension is presented.

There have been found in the recent literature eleven cases of pheochromocytoma with gross or microscopic evidence of malignancy and associated with paroxysmal, malignant, or chronic hypertension. This is contrary to the concept derived from the earlier reviews that malignant pheochromocytomas are not associated with hypertension.

All of these twelve cases showed unilateral involvement, which also is contrary to earlier impressions that malignant pheochromocytomas are always bilateral.

The significance of the chromaffin reaction and the difficulty of histologic differentiation of benign from malignant nature in pheochromocytomas are discussed.

REFERENCES

1. Barlow, H. C.: Malignant tumor with paroxysmal high tension. Clin. J., **76**: 53–58, 1947.
2. Belt, A. E., and Powell, T. O.: Clinical manifestations of chromaffin cell tumors, arising from suprarenal medulla. Surg., Gynec. and Obst., **59**: 9–24, 1934.
3. Bennett, H. S.: Cytological manifestations of secretion in adrenal medulla of cat. Am. J. Anat., **69**: 333–381, 1941.
4. Broster, L. R., and MacKeith, R.: Adrenosympathetic syndrome; chromatrophic tissue tumor with paroxysmal hypertension. Brit. J. Surg., **31**: 393–395, 1944.
5. Buchner, F.: Specific tumors of suprarenal medulla with hypertension; two cases. Klin. Wchnschr., **13**: 617–619, 1934.
6. Cahill, G. F.: Hormonal tumors. Surgery, **16**: 233, 1944.
7. Calkins, E., and Howard, J. E.: Bilateral familial pheochromocytomata with paroxysmal hypertension. J. Clin. Endocrinol., **7**: 475–491, 1947.
8. Edward, D. G. F.: Pheochromocytomata and hypertension. J. Path. and Bact., **45**: 391–403, 1937.
9. Eisenberg, A. A., and Wallerstein, H.: Pheochromocytoma of suprarenal medulla. Arch. Path., **14**: 818–836, 1932.

* We are indebted to Dr. Shields Warren of the New England Deaconess Hospital for permission to review these slides.

10. ERNOULD, H., AND PICARD, E.: Case of sympathetic tumor with paroxysmal arterial hypertension. Rev. belge sc. méd., **6:** 223–251, 1934.
11. ESPERSEN, T., AND DAHL-IVERSEN, E.: Clinical picture and treatment of pheochromocytomas of suprarenal. Acta chir. Scandinav., **94:** 271–290, 1946.
12. EVANS, W. F., AND STEWART, H. J.: Peripheral blood flow in case of pheochromocytoma before and after operation. Am. Heart J., **24:** 835–842, 1942.
13. EWING, JAMES: Neoplastic Diseases. Ed. 4. Philadelphia and London: W. B. Saunders Co., 1160 pp., 1940.
14. KARSNER, H. T.: Human Pathology. Ed. 5. Philadelphia and London: J. B. Lippincott Co., 1013 pp., 1938.
15. KAULBACH, G.: Ein weiterer Beitrag zur Kenntnis der malignen Phäochromozytome. Zentralbl. f. allg. Path. u. path. Anat., **67:** 49–53, 1937.
16. KENYON, A. T.: Cortical tumor; physiologic considerations. Surgery, **16:** 194–232, 1944.
17. KING, E. S. J.: Malignant pheochromocytoma of the adrenals. J. Path. and Bact., **34:** 447–452, 1931.
18. MACKENZIE, D. W., AND MCEACHERN, D.: Tumor of medulla of adrenal (adrenal pheochromocytoma) with removal and relief of paroxysmal hypertension. J. Urol., **40:** 467–476, 1938.
19. MAYOCK, R.L., AND ROSE, E.: Insensitivity to epinephrine in a patient with functioning tumor of the adrenal medulla. Am. J. M. Sc., **213:** 324–330, 1947.
20. MCGAVACK, T. H., BENJAMIN, J. W., SPEER, F. D., AND KLOTZ, S.: Malignant pheochromocytoma of the adrenal medulla. J. Clin. Endocrinol., **2:** 332–338, 1942.
21. MCKENNA, C. M., AND HINES, L. E.: Paraganglioma of suprarenal gland. J. Urol., **34:** 93–96, 1935.
22. MOORE, R. A.: Textbook of Pathology. Ed. 1. Philadelphia and London: W. B. Saunders Co., 1289 pp., 1945.
23. MUNTZ, H. H., RITCHEY, J. O., AND GATCH, W. D.: Adrenalin producing tumor (pheochromocytoma) containing 2300 mg. of adrenalin. Ann. Int. Med., **26:** 133–147, 1947.
24. RABIN, C. B.: Chromaffin cell tumor of suprarenal medulla (pheochromocytoma). Arch. Path., **7:** 228–243, 1929.
25. SNYDER, C. H., AND VICK, E. H.: Hypertension in children caused by pheochromocytoma; report of three cases and review of literature. Am. J. Dis. Child., **73:** 581–601, 1947.
26. SOFFER, L., MENCHER, W. H., AND COLP, R.: Symposium on surgical diagnosis, pheochromocytomata of adrenal gland; report of two cases with operative removal of tumor. S. Clin. North America, **26:** 368–381, 1946.
27. THORN, G. W., HINDLE, J. A., AND SANDMEYER, J. A.: Pheochromocytoma of the adrenal associated with persistent hypertension; case report. Ann. Int. Med., **21:** 122–130, 1944.
28. WASHINGTON, E. L., CALLAHAN, W. P., JR., AND EDWARDS, E. W.: Pheochromocytoma of adrenal medulla; its role in pathogenesis of malignant hypertension. J. Clin. Endocrinol., **6:** 688–698, 1946.
29. WELLS, A. H., AND BOMAN, P. G.: Clinical and pathologic identity of pheochromocytoma. J. A. M. A., **109:** 1176–1180, 1937.

SELECTED ABSTRACTS

Factors Involved in the Experimental Therapy of Metastatic Thyroid Cancer with I^{131}. L. D. MARINELLI, J. B. TRUNNELL, R. F. HILL AND F. W. FOOTE. Radiology, **51**: 553–557, 1948.

Within one year, 9 out of 18 patients with metastatic cancer of the thyroid were selected for treatment with radio-iodine. In only 2 patients tracer doses showed adequate initial retention of the isotope, warranting massive therapeutic doses. One of these cases is presented in detail: To a nearly moribund patient, 60 millicuries of I^{131}, and 7 days later 121 millicuries, were given orally. Some improvement ensued but was interrupted by development of acute thyrotoxicosis, apparently resulting from breakdown of metastatic tumor with release of large amounts of hormone. Elevated values of protein-bound plasma iodine supported the diagnosis. Nine months later another dose of 122 millicuries of the isotope was given; it was preceded by propylthiouracil treatment and no reaction occurred this time. Although neoplastic tissue was still present, about one year after initiation of the treatment the patient had gained 55 pounds and was able to pursue some activities. On the basis of this and similar experiences, the authors recommend the use of radio-iodine in selected cases of metastatic thyroid cancer.

Chicago KURT STERN

Radioactive Iodine Studies of Functional Thyroid Cancer. V. K. FRANTZ, E. H. QUIMBY AND T. C. EVANS. Radiology, **51**: 532–552, 1948.

In a survey of 139 primary carcinomas of the thyroid, followed for at least 5 and up to 17 years, special attention was given to the correlation of histologic type with survival and freedom from disease after adequate treatment. The best results were obtained in neoplasms of the malignant adenoma and of the papillary type, that is, in tumors with the highest degree of differentiation and probably, of functional activity.

Tracer doses of I^{131} were administered to 32 additional patients with thyroid cancer in order to study the isotope uptake by the tumors. Whereas none of the 6 undifferentiated carcinomas in this group showed any uptake of radio-iodine, the isotope did localize in 7 out of 12 malignant adenomas and in 6 carcinomas which presented a mixture of adenomatous and papillary structure. Huerthle cell tumors (benign or malignant) and pure papillary carcinomas failed to take up the isotope.

The uptake of radio-iodine by thyroid cancers is, in addition to the degree of differentiation and functional activity of the tumor, also influenced by the amount and activity of normal thyroid tissue present. Hence tracer doses, as well as therapeutic administration of radio-iodine, may be more effective following thyroidectomy, supplemented in some cases by administration of thyrotropic hormone. On this basis a plan for the use of radio-iodine in selected cases of thyroid cancer is presented by the authors.

KURT STERN

Nitrogen Mustard Therapy for Hodgkin's Disease, Lymphosarcoma, The Leukemias, and Other Disorders. M. M. WINTROBE, AND C. M. HUGULEY. Cancer, **1**: 357–383, 1948.

In a report complete with detailed summary charts, the authors review their experience with nitrogen mustard treatment in 102 patients affected with Hodgkin's disease, lymphosarcoma, acute and chronic leukemia, and a variety of malignancies, including carcinoma of the lung, multiple myeloma and fibrosarcoma. The authors confirm the experience of other investigators and previous reports of their own, that clinical remission occurs mainly in patients with Hodgkin's disease, and to a lesser degree in lymphosarcoma and chronic leukemia. They also emphasize the efficacy of the drug in radiation-resistant cases and note a lack of response in acute leukemia. Disagreeable experiences such as nausea and vomiting, local thrombophlebitis at site of injection, and depressant effect on bone marrow are discussed.

This reviewer agrees that in the dose recommended, namely 0.1 mg./Kg. body weight, danger of inducing hypoplasia of marrow appears to be minimal. Pyridoxine in 100 mg. doses, started the day before a course of treatment and continued for the full period, has proved useful in controlling nausea and vomiting. Local thrombophlebitis may be obviated by injecting the nitrogen mustard through an infusion tube or simply diluting with blood at the time of injection.

Brooklyn LEO M. MEYER

Treatment of the Leukemias. G. L. KAUER. Am. J. M. Sc., **216:** 581–595, 1948.

In a clear, concise, comprehensive review the author carefully summarizes old and new methods in the management of patients with acute and chronic leukemia. He discusses and evaluates such compounds as arsenic, urethane, nitrogen mustard, benzol, folic acid derivatives, urinary extracts, para-aminobenzoic acid and organometallic compounds, and presents the indications for the use of all forms of radiation therapy, giving particular attention to radioactive isotopes. He also discusses symptomatic treatment for bleeding, such as transfusion, penicillin, gel foam and toluidine blue. This paper is recommended to all those who are interested in the treatment of leukemia. A substantial bibliography is included.

LEO M. MEYER

The Histological Effects of Nitrogen Mustard on Human Tumors and Tissues. S. SPITZ. Cancer, **1:** 383–398, 1948.

The author presents histologic data on biopsy and autopsy material obtained from 57 fatal cases of Hodgkin's disease, lymphosarcoma (including reticulum cell sarcoma), lymphocytic and myelocytic leukemia, and a variety of malignant neoplasms, treated with nitrogen mustard. The changes observed, particularly in lymph nodes, during varying periods of time after administration of the drug, are well depicted in a series of photomicrographs. Of particular interest are the descriptions and photographs of severe hypoplasia of the bone marrow and testes following HN_2 therapy. The author confirms previous reports of the particular efficacy of this drug in Hodgkin's disease and summarizes the pathologic changes. Less striking but encouraging results were obtained in the group of patients with reticulum cell sarcoma. No cellular changes were noted in malignant tissues from patients with epithelial tumors. No alterations were found in the adrenal glands or mucosa of the gastrointestinal tract.

LEO M. MEYER

Bullet Embolism: A Case of Pulmonary Embolism Following the Entry of a Bullet into the Right Ventricle of the Heart. D. H. COLLINS. J. Path. and Bact., **60:** 205, 1948.

In the teaching of embolism, mention is always made of foreign bodies in the circulation. Collins points out that only a single paper (Straus, R.: Arch. Path., **33:** 63, 1942) on solid metallic objects free in the channels of the cardiovascular apparatus has been published in recent years. He reviews the literature on the subject, which describes a total of only 8 cases, and adds one of his own. With the aid of a homemade device a 38 year old man fired a Sten gun bullet, at very low velocity, into his chest, and then walked away to seek help. On the only x-ray films made the bullet was found to be intracardiac, moved with the heart beat, but did not whirl about. Eight days after injury, exploratory operation of the pericardium and heart wall did not disclose the bullet; an infected left hemothorax was drained. Death came 11 days following operation or 19 days after the attempted suicide.

At necropsy, it was learned that the bullet had entered the right ventricle just to the right of the interventricular sulcus at the base of the conus arteriosus. There was a deep ulcer on the right side of the interventricular septum. The missile was not found in the heart which was elsewhere normal.

In the left lung there was a wound of the anterior border from which bleeding had produced the subsequently infected hemothorax. The bullet, which sometime after the pa-

tient's admission to the hospital freed itself from its bed in the interventricular septum, was found in the lower primary branch of the right pulmonary artery; the tip of the bullet pointed distally. Two lower pulmonary lobes were infarcted, as a result.

Fort Wayne, Indiana S. M. RABSON

The Effect of Castration on the Action of Some Barbiturates. G. R. CAMERON, G. H. COORAY, AND S. N. DE. J. Path. and Bact., 60: 239, 1948.

More than 10 years ago the senior author noted that spayed adult rats were more susceptible than normal female rats to pentobarbital sodium. The work was resumed after the war. One-half of 40 male Wistar rats were castrated, while on the other 20 animals only laparotomy was performed. The experiments were begun after complete healing in all animals, and extended over 225 days.

It was found that the castrates had a lowered tolerance for the quick acting barbiturate (pentobarbital sodium) but not for the slow acting barbiturate (barbital sodium). The administration of testosterone was followed by improved tolerance. In the control series aging was accompanied by progressive lowering of tolerance which was not marked.

Cameron and his co-workers suggested that this disturbed barbiturate action indicates an altered functional relationship between the gonads and the liver. They referred to the work of others, including the Biskinds, who discussed the inactivation of sex hormones by the liver, and offered the hypothesis that these hormones are employed as adjuvants or co-enzymes in liver detoxication.

S. M. RABSON

Function and Arrangement of Bacterial Flagella. T. Y. KINGMA BOLTJES. J. Path. and Bact., 60: 275, 1948.

Bacterial flagella were described by Ehrenberg in 1838, and later in 1872 (Cohn), 1877 (Koch), and 1897 (Migula). Forty-years after Ehrenberg, van Tieghem argued that they were not locomotive appendages but artefacts. Pijper has recently revived the controversy and some of his arguments may be read in J. Path. and Bact. (58: 325, 1946). Boltjes has entered the arena not only to challenge Pijper's assertions of 1930 and 1931–32 that flagellation does not exist, but that flagella are staining artefacts. Both papers must be read to appreciate the vivacity of the arguments and the evidence mustered to support them. Boltjes' paper has some of the clearest electron photomicrographs this reviewer has yet seen reproduced.

S. M. RABSON

The Weltmann Reaction in Infantile Tuberculosis. GUSTAVO CARDELLE, ANTONIO SELLEK and ALEJANDRO DEL FRADE. Revista Cubana de Laboratorio Clinico, 2: 165–179, January-March, 1948.

The Weltmann reaction was used in 47 cases of infantile tuberculosis of different clinical types. In 90 per cent of inactive cases the reaction was normal. In 80 per cent of primary pulmonary lesions the reaction recorded a shift to the left, generally in tubes five or four.

In cases of primary pulmonary or meningeal lesions which were treated (sulfonamides or streptomycin), the reaction returned progressively to normal as the patient improved clinically. The reaction, therefore, is useful in evaluating the activity of tuberculous lesions, their exudative tendency and the role of coincidental or intercurrent disease which might modify the picture.

Cincinnati WILLIAM MCKEE GERMAIN

The Kline Cardiolipin Reaction in the Serodiagnosis of Congenital Syphilis. ANTONIO SELLEK, ALEJANDRO DEL FRADE and ALBERTO MARTINEZ. Revista Cubana de Laboratorio Clinico, 2: 186–190, January-March, 1948.

An evaluation of the Kline test with cardiolipin antigen was made on 910 serums (910 cases) in congenital syphilis. Parallel control tests were done using the standard Kahn,

the Meinicke (MKR II), the Mazzini (F) and the Sellek II antigens. Of the 910 serums, 81 were positive with one or more of the tests used. Of the 81 positives, the Kahn reaction was positive in 70 per cent; the Meinicke in 70 per cent, the Mazzini in 72.7 per cent and the Kline (cardiolipin) in 76.5 per cent. Of the positive serums 22 per cent were so recorded on the basis of a single positive test.

The principal virtues of the Kline cardiolipin reaction, apart from its sensitivity, are (1) the easy reading of the results, (2) the practical absence of doubtful cases, and (3) the scant number of positives of one plus intensity.

WILLIAM MCKEE GERMAIN

Pseudohemophilia: Report of a Case Simulating Acute Appendicitis. George L. Calvy. U. S. Naval Med. Bull., **48**: 616–620, 1948.

An eighteen year old white male had experienced several episodes of acute abdominal pain, during one of which he was hospitalized and prepared for surgery. However, he underwent marked rapid improvement and surgery was not performed. While on board ship, the patient developed a cramping, para-umbilical pain on arising, and became nauseated. By noon, the pain had shifted to the right lower quadrant of the abdomen and malaise was prominent. On examination, there was point tenderness in the right lower quadrant, and the spleen was palpable. No petechiae or ecchymoses were seen. During routine pre-operative laboratory tests, a bleeding time of 7¼ minutes (Duke) was noted; the technician experienced great difficulty in stopping the blood flow from the ear puncture.

Surgery was again postponed and a more thorough workup begun. This disclosed microscopic hematuria, normal coagulation time (Burker) with a non-retractable clot, prolonged bleeding time, normal platelet count, and a positive capillary fragility test (Rumpell-Leeds).

Family history revealed that a sister had died suddenly from a "blood clot on the brain". Allergic, rheumatic, and hemorrhagic disease was denied.

The patient was treated conservatively and with blood transfusion, and made complete recovery. The author believed that the abdominal pain was due to hemorrhage into the mesenteric root. He also discusses the entity known as pseudohemophilia (von Willebrand type).

Cleveland BEN FISHER

The Effect of Heparin and Dicumarol Anticoagulant Therapy Upon the Erythrocyte Sedimentation Rate. STUART W. COSGRIFF. J. Clin. Investigation, **27**: 435–438, 1948.

In spite of the frequent reports of use of heparin and Dicumarol, there is no unanimity in the literature as to the effect of these drugs on the erythrocyte sedimentation rate *per se*. Since the sedimentation rate is a test of some importance in the diagnosis, prognosis, and treatment of certain diseases in which anticoagulants are used, it was felt that some definitive information would be necessary.

Dicumarol was given orally to ten subjects with stabilized sedimentation rates. Clinical doses were given, beginning with 300 mg. on the first day. Heparin was administered intravenously in 50 mg. doses. Sedimentation rates (Westergren) were determined before giving the medication, 30 minutes after the heparin, and at several later intervals.

It was found that in the dosages employed clinically, heparin and Dicumarol did not significantly alter the erythrocyte sedimentation rate. The test can be used concurrently with anticoagulant therapy in the prognostic and therapeutic evaluation of thrombotic conditions.

BEN FISHER

BOOK REVIEWS

Bronchogenic Carcinoma and Adenoma. With a Chapter on Mediastinal Tumors. By B. M. FRIED, M.D., Associate Attending Physician, Montefiore Hospital for Chronic Diseases, New York. 306 pp., 118 figs., 19 tables. $6.00. Baltimore: The Williams & Wilkins Company, 1948.

This book is, in a sense, a revision of the author's monograph on this subject entitled *Primary Carcinoma of the Lung*, published in 1932. However, no reference is made in the preface to this previous contribution and Fried has chosen not to offer this book as a second edition of his previous one.

The first one hundred and five pages are devoted to a general basic consideration of the subject in which the author's previous extensive investigations in pulmonary diseases are well represented. This section consists of four chapters and includes pathologic features and classification, etiology and metastasis. The prevalence of primary pulmonary cancer and the apparent versus the real increase of this disease are thoroughly discussed, with the author expressing the opinion that in all likelihood the increase has been more apparent than real.

Chapters V and VI deal with clinical manifestations and here the author quite admirably and forcibly calls attention to the atypical clinical features so often present: the silent or "inapparent" stage, often of considerable duration, the necessity for recognizing and interpreting early manifestations and the realization that recognition only of typical symptoms usually results in the diagnosis of a lesion which is already untreatable.

Chapters VII and VIII are entitled Superior Pulmonary Sulcus Tumors and Hypertrophic Pulmonary Osteoarthropathy. Of the latter, the author reviews the existing unsatisfactory theories of pathogenesis, expresses the interesting viewpoint that a disturbance in the function of organs of internal secretion might be responsible and suggests that the cause may not be remote from that of acromegaly, and that pituitary dysfunction might be a factor.

Chapter IX, consisting of seventeen pages, deals with radiographic and histologic diagnosis and, in the next chapter, radiation therapy and surgical treatment are briefly discussed.

The chapter on bronchiogenic adenoma gives a very good review of the nature and modern concept of this interesting tumor and presents objectively the controversy between those who believe that this is a benign neoplasm and those who consider it potentially or actually malignant. The author appears to agree with the latter and states, "One no longer considers that the treatment of the visible segment of the adenoma is sufficient alone to cure the disease."

The last chapter presents a comprehensive discussion of mediastinal tumors.

The author has inserted three short supplements. The one dealing with alveolar cell cancer and adenomatosis of the lungs calls attention to the similarity of these two lesions in humans to epizootic adenomatosis in sheep and the possibility that the latter is caused by a filterable virus. Supplement II considers bronchiogenic cancer combined with pulmonary tuberculosis and consists entirely of illustrative case histories with no general discussion of possible etiologic relationship. The third supplement consists of a brief discussion of bronchiogenic mesodermal tumors in which chondroma and leiomyoma are discussed.

This book provides the reader with a thorough and authoritative presentation by a competent observer of a subject of primary importance in the field of oncology. All phases are faithfully presented. The bibliography is complete and the summaries and comments frequently found at the end of a chapter are a valuable feature.

Detroit OSBORNE A. BRINES

Education for Professional Responsibility. A 203 page report of the "Inter-Professions Conference on Education for Professional Responsibility" held at Buck Hills, Pennsyl-

vania, April 12, 13, and 14, 1948. Pittsburgh: The Carnegie Press, Carnegie Institute of Technology, 1948.

As stated in the Foreword, the subject matter of the Conference was confined to five professions, represented by teachers from schools of divinity, medicine, law, engineering, and business. Owing to the widespread and stimulating interest in the Conference, the formal papers were published in the present book form. There is appended a three page list of brief bibliographies on professional education.

The report of the first session of the Conference comprises eight papers on the Objectives of Professional Education. The second session comprises six papers on Contents and Methods while the third session consists of five papers devoted to Social and Humanistic Aspects of Professional Education.

The publication of these interesting papers side by side in book form is of considerable advantage to the reader. There is evidently an awareness among teachers in our professional schools that all is not well with our far-flung complex society, and that the impact of war without peace is bringing society's illness into bold relief. It is apparent that the problem of social and public responsibility in professional education has been neglected and must, henceforth, receive more attention.

Objectives, curricula, and methods in teaching are roundly criticized by teachers themselves, yet there is a wholesome constructiveness throughout their criticism which has impressed this reviewer very favorably. The reader who is perchance looking for something to sell will acquire nothing from this timely collection of papers, but he should gain a very desirable insight into, and perspective of, modern professional education, not only in action but under fire.

Davenport, Iowa F. H. LAMB

Manual of Veterinary Clinical Parasitology. By EDWARD A. BENBROOK, V.M.D., Professor and Head of the Department of Veterinary Pathology, Iowa State College and MARGARET W. SLOSS, B.S., D.V.M., M.S., Assistant Professor of Veterinary Pathology, Iowa State College, Ames, Iowa. 187 pp., 243 figs. $4.50. Ames, Iowa: The Iowa State College Press, 1948.

The book is divided into three sections, the first of which deals with examination of feces of domestic animals and fowl for parasitism. There is a detailed description of the technic, followed by eighty pages of excellent photomicrographs depicting the ova and larvae of such parasites as are likely to be encountered and certain objects which may be mistaken for parasites by the untrained. The second section describes the methods used in the examination of the skin for evidence of parasitic mites. Following a brief classification, there is a short description of the more important species and again excellent illustrations, chiefly photomicrographs, although there are some good line drawings illustrating points of differentiation of the species. The third section is entitled The Diagnosis of Lice Infestations. There is a list of the species and their hosts and ten pages of illustrations. There are twenty-one pages of references.

While the book is obviously intended for the student and practitioner of Veterinary Medicine, this reviewer is glad to have it in his library for he, like many clinical pathologists, has occasion to give aid to the veterinarian.

Rochester, New York W. S. THOMAS

Symposia on Nutrition of the Robert Gould Research Foundation. Volume 1. Nutritional Anemia. Edited by ARTHUR LEJWA. 194 pp., 73 figs., 10 tables. Cincinnati: The Robert Gould Research Foundation, Inc., 1947.

The book is a collection of papers prepared for the Symposium on Nutritional Anemia organized by the Robert Gould Research Foundation and held in Cincinnati in October 1947. It includes works on the physiologic aspects of anemia, treatment of nutritional anemia with emphasis on the use of folic acid, iron, vitamin B complex and vitamin C, as well as discussions of the metabolism of folic acid, iron and copper. Some of the material is of more interest to the clinician than the pathologist, although readers especially con-

cerned with hematology will find all the papers informative. Two articles may be singled out as being of particular interest to pathologists. The discussion of the megaloblastic anemia syndrome of infancy by Wolf W. Zuelzer is not only an important addition to the hematologic literature but is also an excellent example of the significant contribution which a pathologist can make in the field of clinical research. Perhaps the most useful paper is the review of the clinical and physiologic aspects of the anemic state presented by Maxwell M. Wintrobe. This can be read with profit by all pathologists desiring an authorative background of information on anemias in general, as seen from the clinician's point of view and, as such, offers a concise and interesting source of invaluable data which would be of help in the preparation of discussions of cases in which anemia is a factor contributing to the clinicopathologic picture.

Detroit LAWRENCE BERMAN

BOOKS RECEIVED

Abderhalden, R.: Medizinische Terminologie, Wörterbuch der Gesamten Medizin und der Verwandten Wissenschaften. 1213 pp. Basel: Benno Schwabe and Company (imported by Grune and Stratton, Inc., New York), 1948.

Burnhand, R., Jaeger, H., Amsler, M., Verrey, F., Martin, E., Nicod, J. C., and Hauduroy, P.: Le Problème des Tuberculoses Atypiques. Étude Clinique, Étude Anatomique, Étude Bacteriologique. 435 pp., 20 figs. 760 francs. Paris: Masson & Cie., 1946.

Ciferri, R., et Redaelli, P.: Mycopathologia. Vol. IV., Fasc. 2. Amsterdam: Uitgeverij, Dr. W. Junk, 1948.

Felsenfeld, O., Young, V. M., and Conner, P.: Relationes de Avctis Scientiis Tempore Belli A. 1939–1945. 75 pp.

Fosdick, R. B.: The Rockefeller Foundation. A Review for 1947. 64 pp., 8 illus. New York: Rockefeller Foundation, 1947.

Friedman, M.: Functional Cardiovascular Disease. 266 pp., 24 tables. $3.00. Baltimore: The Williams & Wilkins Co., 1947.

Leitner, St. J., and De Dulanto, F.: La Enfermedad de Besnier-Boeck-Schaumann. 240 pp., 37 figs., 15 tables. Barcelona: Collecion Espanola de Monografias Medicas, 1946.

Pinck, L. A.: A Biochemical Hypothesis of the Genesis of Cancer. 18 pp. Annals of the New York Academy of Sciences, Vol. 50, Art. 1, February 6, 1948.

Ricci, J. V.: Diagnosis in Gynaecology. A Classification of Gynaecological Diseases Based on Actiology and the Clinical Logic for Diagnosis. 259 pp. $4.50. Philadelphia and Toronto: The Blakiston Company, 1948.

Schweizerische Zeitschrift für Pathologie und Bakteriologie, Revue Suisse de Pathologie et de Bacteriologie. Vol. XI, 1948. New York: S. Karger.

Smith, L. W., and Gault, E. S.: Essentials of Pathology. Ed. 3. 764 pp., 740 figs. $12.00. Philadelphia: The Blakiston Company, 1948.

Sorba, M.: Gonadotrophines et Tumeurs Testiculaires. 80 pp., 2 figs., 5 tables. 6 broschiert francs. Basel: Benno Schwabe and Company, 1946.

Wernly, M., et Berdjis-Chamsi, Ch.: Les Parathyroïdes Humaines Contribution á L'Étude des Hyperplasies et des Adénomes. 144 pp., 44 figs., 13 tables. 10 broschiert francs. Basel: Benno Schwabe and Company, 1947.

TECHNICAL SECTION

A METHOD OF DETERMINING THE DISTRIBUTION OF ERYTHROCYTE FRAGILITY IN HUMAN BLOOD*

E. E. ECKER, PH.D., C. W. HIATT, PH.D., AND L. M. BARR, B.S.

From the Institute of Pathology, Western Reserve University, and the University Hospitals Cleveland, Ohio

Although erythrocyte fragility tests of the type described by Todd and Sanford[3] are commonly used to aid in the diagnosis of red cell dyscrasias, the methods do not provide a high order of accuracy. Such tests also fail to provide any information about the distribution of fragility among the red cell population.

The use of a photoelectric colorimeter for the measurement of percentage of hemolysis, as described below, gives a considerable increase in accuracy of the method. In this method the data are arranged in such a manner that the final record obtained in each test is a Fragility Distribution Plot which represents approximately the variation in resistance to hypotonic lysis among the cells of the population examined.

METHOD

Reagents

A stock solution of 0.85 per cent sodium chloride is prepared by dissolving 8.5 Gm. of C.P. NaCl (dried to constant weight) in distilled water to a total volume of one liter.

Dilutions of the stock solution are made, as indicated in Table 1, to provide twelve hypotonic salt solutions of regularly varying salt concentration. It is convenient to use two 100 ml. burets in the preparation of the dilutions, one buret being used for the stock salt solution, the other for the distilled water diluent. The dilutions should be prepared with great care and stored in dried, tightly stoppered bottles.

Blood samples are collected by allowing 5 ml. of the patient's blood to flow directly from the syringe into 1 ml. of 1.4 per cent sodium oxalate solution in a 10 ml. test tube. The tube is stoppered and agitated gently to effect thorough mixing.

The erythrocytes are sedimented by centrifugation at low speed (1000 r.p.m.) and washed twice in 10 ml. of 0.85 per cent saline to remove the plasma. The washed cells are then resuspended in 0.85 per cent saline to such concentration that, when 1 ml. of the suspension is mixed with 4 ml. of distilled water, complete hemolysis results and the clear solution yields a reading of 300 ± 10 on the Klett-Summerson Photoelectric Colorimeter using the green (# 54) filter.

Procedure

Twelve clean, dry colorimeter tubes are assembled for each cell suspension to be tested. The tubes are numbered from 1 to 12, and 4 ml. of the corresponding

* Aided by a grant from The Commonwealth Fund, New York, N. Y.
Received for publication, September 10, 1948.

diluted saline solution (see Table 1) is placed in each tube. A separate pipet is used for each of the twelve solutions to avoid errors of dilution. Then 1 ml. of the prepared cell suspension is added to each tube. The contents of the tubes are mixed thoroughly and allowed to incubate for 15 minutes at 37 C.

The tubes are then centrifuged for 5 minutes at 1500 r.p.m. to sediment the unhemolyzed cells. Each tube is placed in succession in the colorimeter, and a colorimeter reading of the clear supernatant fluid is obtained. The readings are recorded on a mimeographed data sheet of the type shown in Figure 1.

Each colorimeter reading (column 3) is subtracted from the reading directly above it, and the difference is entered in column 4 opposite the lesser reading.

The Fragility Distribution Plot is made on the chart below (Figure 1) by entering a point on the chart for each tube. The ordinate of each point is the value

TABLE 1

PREPARATION OF HYPOTONIC SALINE SOLUTIONS

SOLUTION NO.	ML. 0.85% NaCl	ML. DISTILLED WATER
1	15.5	84.5
2	22.8	77.2
3	30.0	70.0
4	37.3	62.7
5	44.7	55.3
6	52.0	48.0
7	59.4	40.6
8	66.7	33.3
9	74.1	25.9
10	81.5	18.5
11	88.8	11.2
12	96.2	3.8

of the colorimeter reading difference, and the abscissa is the Plot Number listed in column 5. The points are connected by a series of straight lines to complete the graph.

DISCUSSION

In the procedure outlined above it will be seen that plotting the colorimeter reading difference against salt concentration yields an approximate expression of the first derivative of the curve of per cent of hemolysis versus salt concentration. The Fragility Distribution Plot is essentially an approximation of the distribution of resistance to lysis. The vertical axis of the graph represents, in arbitrary units, the rate of change in hemolysis, and the horizontal axis represents the total salt concentration.

The concentrations of the twelve hypotonic saline solutions used have been chosen so that when 4 ml. of each solution is mixed with 1 ml. of the suspension of cells, the over-all salt concentrations are those listed in column 1, Figure 1. Allowance has been made for the volume occupied by the cells themselves by application of the following formula, which is based upon the assumption that

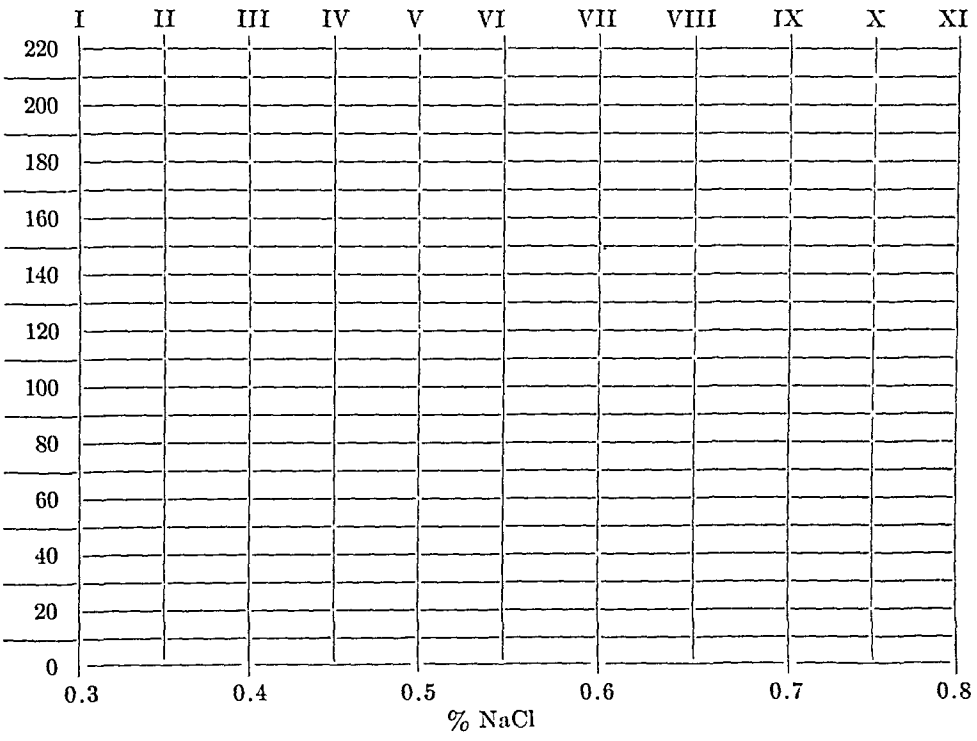
FIGURE 1

FORM OF THE DATA SHEET FOR OBTAINING THE FRAGILITY DISTRIBUTION PLOT

Patient:..... Age:..... Sex:..... Date:.....

R.B.C. Count:..... x 10^6/mm^3. Hemoglobin:.....Gm./100 ml. of blood

Tube No.	% NaCl	Color Rdg.	Color Diff.	Plot No.
1	0.275			
2	0.325			I
3	0.375			II
4	0.425			III
5	0.475			IV
6	0.525			V
7	0.575			VI
8	0.625			VII
9	0.675			VIII
10	0.725			IX
11	0.775			X
12	0.825			XI



the prepared cell suspension contains approximately one per cent by volume of cells:

$$\text{Over-all NaCl concentration } (\%) = \left(\frac{v + 0.99}{4.99}\right)(0.85)$$

where v = ml. of 0.85 per cent NaCl solution contained in 4 ml. of the dilute solution (see Table 1).

The method described above is proposed as a valuable technic for routine clinical use. To illustrate the type of result obtained with the method in this laboratory, the examples shown in Figures 2 and 3 are presented.

Figure 2 is a composite diagram of the Fragility Distribution Plots obtained for six ostensibly normal bloods. The symmetry of the curves is consistent with

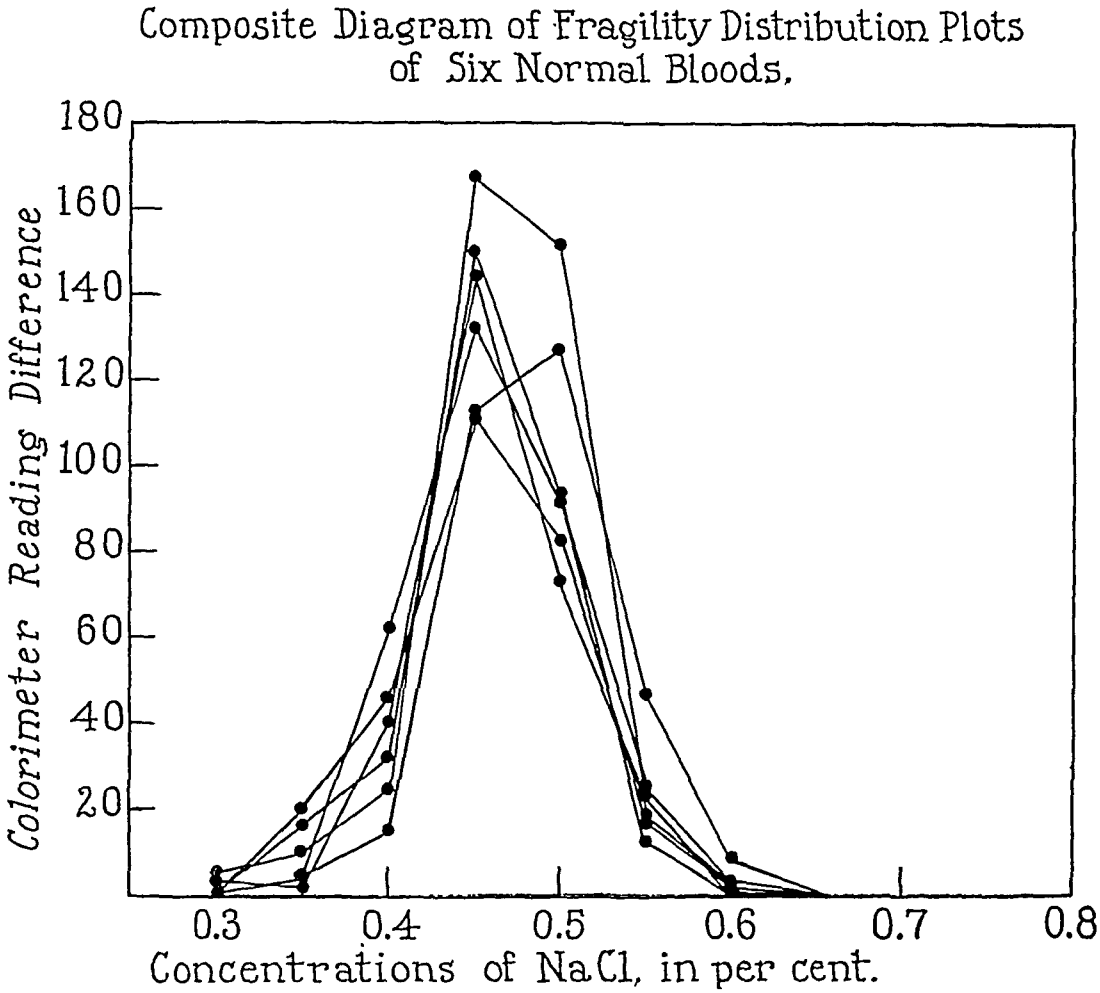


FIG. 2

the early observation of Madsen[2] that the resistance to lysis among the cells of any monodisperse population should follow the normal Gaussian distribution curve. The plots for the six normal specimens reported here serve to delineate a narrow range for normal variation, the center of area of each curve occurring between 0.46 and 0.48 per cent NaCl. Sufficient samples have not yet been examined to designate completely the normal range of variation, but the data presented in Figure 2 justify the conclusion that a fairly well defined normal range exists.

In a series of abnormal bloods studied, striking differences in the position, breadth, and symmetry of the Fragility Distribution Plots have been noted. Of particular interest is the unusual type of distribution found to occur in the several cases of pernicious anemia studied. Four of these plots are reproduced in Figure 3. These plots seem to represent biphasic distribution curves, suggest-

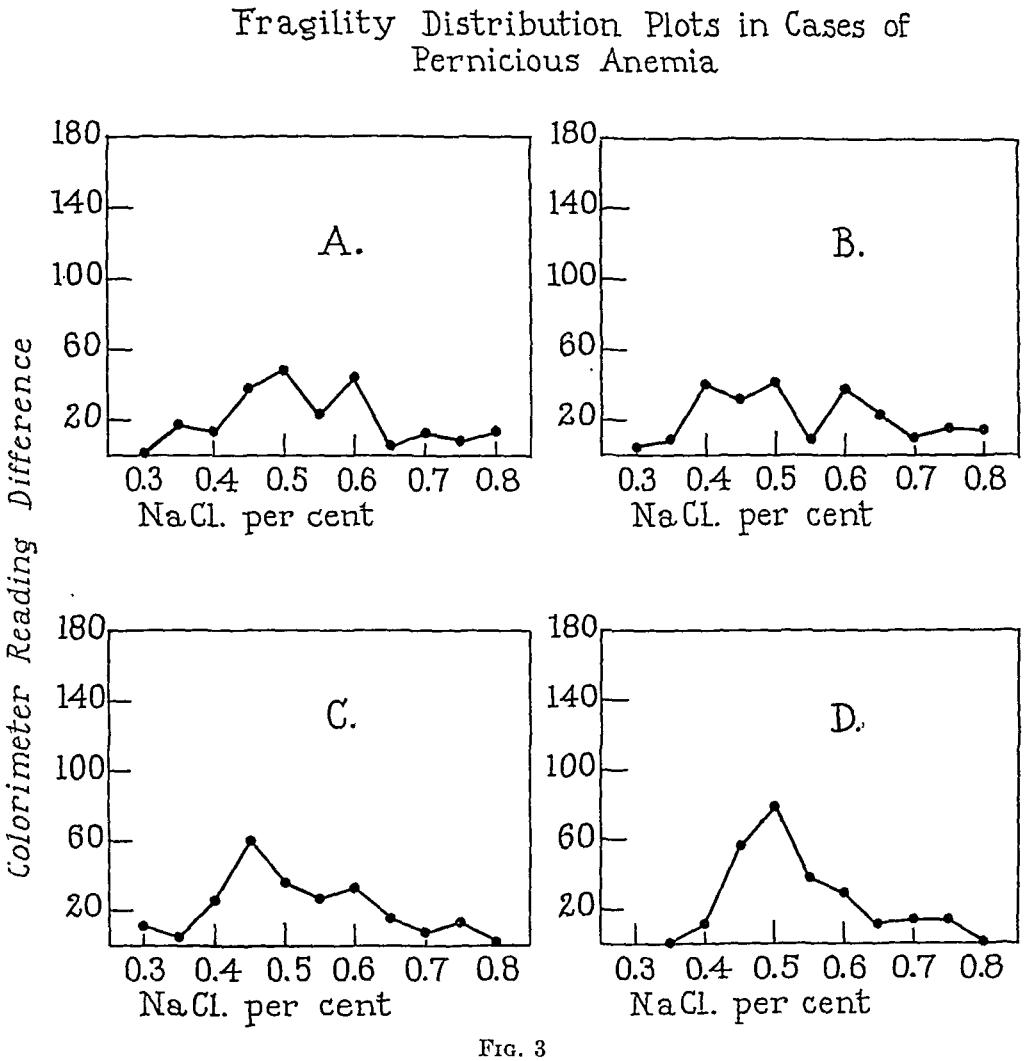


FIG. 3

ing the presence of two distinct types of cells, discernible on the basis of relative hypotonic fragility. In the plot shown in Figure 3D, the curve is not distinctly biphasic, but shows an incipient peak in the approximate location of the secondary peaks of the other curves in the series. In this case, the patient had recently been found to have pernicious anemia, and therapy had just been commenced. The other plots represent cases of longer duration with prolonged therapy.

From the work of Buckman and MacNaugher,[1] who showed that the reticu-

lated erythrocytes in cases of pernicious anemia were abnormally fragile, it is not unreasonable to assume that the secondary peak represents reticulocytes. Further work is necessary to verify this assumption.

The correlation of the test with other clinical data obtained for normal and pathologic bloods, including serial studies of patients under treatment, is a necessary prerequisite to the standardization of the method. When sufficiently amplified in terms of clinical data, the method should prove to be valuable in the differential diagnosis of anemias and in following cases under treatment.

SUMMARY

Details are given of a method for determining the approximate distribution of fragility among the erythrocytes of a given population. The results are expressed in the form of a Fragility Distribution Plot which, by its position, breadth, and symmetry, represents the condition of the red cells with respect to fragility.

The Fragility Distribution Plots obtained in four cases of pernicious anemia occur as biphasic distribution curves, indicating the presence of two types of red cells, one type distinctly more fragile than the other.

The method is proposed as one which, after a more thorough correlation with clinical data, may be expected to be useful in the diagnosis and treatment of anemias.

REFERENCES

1. Buckman, T. E., and MacNaugher, E.: The relative fragility of reticulated red corpuscles. J. Med. Research, **44**: 61–72, 1923–24.
2. Madsen, Th.: Ueber Tetanolysin. Ztschr. f. Hyg., **32**: 214–238, 1899.
3. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods. A Working Manual of Clinical Pathology. Ed.10. Philadelphia: W. B. Saunders Company, 911 pp., 1943.

TITRATION OF PHYSIOLOGIC AND IMMUNE ANTIBODIES WITH WASHED AND UNWASHED ERYTHROCYTES*

J. GUREVITCH, M.D., Z. POLISHUK, M.D., AND D. HERMONI, M.Sc.

From the Department of Bacteriology and Serology and the Department of Obstetrics and Gynecology of the Rothschild-Hadassah University Hospital, Jerusalem, Palestine

Distinct differences in the behavior of natural and immune antibodies have been reported. Studies on the influence of temperature on the titer of iso-agglutinins have shown that the titers of the serums examined were highest at low temperatures, gave distinct reactions at 37 C., and were lowest at high temperatures. Irregular agglutinins, however, were found to act best at higher temperatures.[6] The early observations on the behavior of Rh agglutinins[5] led to the conclusion that they act best at 37 C. and were, therefore, called "warm" agglutinins. Further studies on the thermal range of anti-Rh agglutinins and iso-agglutinins have confirmed the above observations.[2] Another distinction between immune and physiologic agglutinins was found when these were titrated in human serum[1] and different albumin-containing mediums.[4] While the use of serum, as a diluent in titration, enhanced the action of immune agglutinins, it had no influence on the titers of physiologic antibodies.

In the course of earlier studies on the mechanism of the conglutination technic in titrating Rh antiserums, we made the observation of distinct differences in behavior of physiologic and immune antibodies, when titrated with washed and unwashed erythrocytes. The following is a report of a study on this subject.

MATERIALS AND METHOD

Twenty-three normal serums and 15 immune serums were used for this experiment. Of these latter, 6 serums were obtained from Rh-negative women with signs of anti-Rh immunization, 7 from women with signs of immunization to A or B antigens, and two were anti-Rh test serums. The serums were titrated by serial dilution in saline. To each tube in the first row, one drop of 2 per cent unwashed erythrocyte suspension was added and to each tube in the second row one drop of 2 per cent suspension of erythrocytes which had had three washings in saline. Reading of results was made for the anti-Rh serums after incubation of one hour at 37 C. and for the anti-A and anti-B serums after one hour at room temperature. The final titer was checked both macroscopically and microscopically. A difference of at least two test tubes in titration by serial dilution was considered as significant.

RESULTS

The results obtained by the titration of physiologic antibodies in normal saline (Table 1) are uniform and clear-cut. No differences were found between the titers of physiologic agglutinins with washed and unwashed erythrocytes.

* Received for publication, September 30, 1948.

TABLE 1

TITRATION OF NORMAL SERUMS WITH WASHED AND UNWASHED ERYTHROCYTES

SERUM NUMBER	GROUP	TITER WITH UNWASHED ERYTHROCYTES	TITER WITH WASHED ERYTHROCYTES
1	A	1:16	1:16
2	A	1:16	1:16
3	A	1:32	1:32
4	A	1:4	1:8
5	A	1:8	1:8
6	A	1:32	1:64
7	A	1:16	1:32
8	A	1:64	1:64
9	A	1:8	1:16
10	A	1:32	1:16
11	A	1:64	1:64
12	A	1:16	1:16
13	B	1:32	1:32
14	B	1:16	1:32
15	B	1:8	1:8
16	B	1:16	1:16
17	B	1:32	1:32
18	B	1:16	1:16
19	B	1:16	1:16
20	B	1:16	1:16
21	B	1:8	1:16
22	B	1:16	1:32
23	B	1:64	1:64

TABLE 2

TITRATION OF IMMUNE SERUMS WITH WASHED AND UNWASHED ERYTHROCYTES

SERUM NUMBER	AGGLUTININS	TITER WITH UNWASHED ERYTHROCYTES	TITER WITH WASHED ERYTHROCYTES
1	anti-A	1:128	1:32
2	anti-A	1:64000	1:2000
3	anti-A	1:8000	1:512
4	anti-A	1:4000	1:512
5	anti-A	1:4000	1:1000
6	anti-B	1:1024	1:64
7	anti-B	1:128	1:32
8	anti-Rh	1:32	1:1
9	anti-Rh	1:16	1:2±
10	anti-Rh	1:16	1:4
11	anti-Rh	1:16	1:16
12	anti-Rh	1:64	1:64±
13	anti-Rh	1:2	1:4±
14	anti-Rh	1:1	1:1
15	anti-Rh	1:2	1:2

The results of titration of 15 immune serums are summarized in Table 2. Distinctly lower titers of the immune antibodies were established in 10 serums

when titrated with washed erythrocytes, as compared with the results obtained in the titration with unwashed erythrocytes. No differences in titer were observed in 5 of the immune serums, all of which were anti-Rh serums. In 3 of the anti-Rh serums the titer obtained with unwashed erythrocytes was low (1 or 2 units). Two of these anti-Rh serums were test serums.

DISCUSSION

The results of titration of immune and physiologic antibodies with washed and unwashed erythrocytes seem to present an additional method for differentiating these two biologically different antibodies.

In a perusal of the literature, an early study by Hamburger[3] was found on the agglutinability and absorptive capacity of washed erythrocytes. This author could not find any differences in the agglutinability of normal red blood cells, when examined with normal A or B serums, even after 10 washings in normal saline. Although in trying to determine the properties of the different antibodies in serums we started from a different angle, our results corroborate the findings of Hamburger. There is no need for 10 washings, inasmuch as 3 consecutive washings with saline are safe enough to produce erythrocytes that are free of serum proteins.

The results of our studies with physiologic and immune anti-A and anti-B serums point to a distinct difference in property of physiologic and immune antibodies. No differences could be found in the titration of physiologic antibodies when performed with unwashed or washed erythrocytes, while distinct differences in the results of titration of the immune serums were observed. These differences were especially noticeable in the titration of the anti-A and anti-B immune serums, in which the original titer was much higher than in the anti-Rh immune serums. In reading the results of titration, a difference of two tubes was considered as significant. In the titration of the anti-A and anti-B serums, differences of 3, 4 and up to 6 tubes were found. Of the 8 anti-Rh serums, 3 gave significantly higher titers with unwashed erythrocytes than with washed erythrocytes. In 5 anti-Rh serums, no differences in titer could be found in tests with washed and unwashed erythrocytes.

One of two possibilities must be considered in seeking an explanation for the above observation: (1) The washing of the erythrocytes reduced the agglutinability of the red blood cells, or (2) the washing of the erythrocytes frees them from some of the serum proteins present in the suspension of the erythrocytes. The results of titration with physiologic antibodies, as well as the above-mentioned studies by Hamburger, prove that the agglutinability of the erythrocytes is not reduced by several or many consecutive washings.

As has already been observed, the presence of serum proteins or albumin distinctly enhances the action of immune antibodies. This is apparently the basis for the conglutination reactions. It would thus seem, in titrations with immune antiserums, that the presence of serum proteins in unwashed erythrocytes is the cause of the higher titers observed with unwashed cells as compared with tests made with washed erythrocytes (Table 2). The titration

of antibodies with washed and unwashed erythrocytes presents another possible method for differentiating between physiologic and immune antibodies.

SUMMARY

1. The results of a study on the titration of physiologic and immune antibodies with unwashed and washed erythrocytes are reported.

2. Twenty-three normal serums and 15 immune serums were used in the study. Whereas physiologic antibodies gave no differences in titration when tested with unwashed and with washed erythrocytes, immune antibodies showed distinctly higher titers when titrated with unwashed erythrocytes than with washed red blood cells.

3. The results indicate a new method of differentiating immune and physiologic antibodies.

REFERENCES

1. Boorman, K. E., Dodd, B. E., and Morgan, W. T. T.: Enhancement of action of immune haemagglutins in human serum. Nature, **156**: 663–664, 1945.
2. Davidsohn, I.: Rh antibodies. Am. J. Clin. Path., **15**: 95–105, 1945.
3. Hamburger, C.: Untersuchungen über die Agglutinabilität und Absorptionsfähigkeit gewaschener Blutkörperchen. Acta path. et microbiol. Scandinav., **7**: 171, 1930.
4. Levine, P.: The present status of the Rh factor. Am. J. Clin. Path., **16**: 597, 1946.
5. Levine, P., Katzin, E. M., and Burnham, L.: Atypical warm isoagglutinins. Proc. Soc. Exper. Biol. and Med., **45**: 343, 1940.
6. Wiener, A. S.: Blood Groups and Transfusions. Springfield, Ill.: Charles C Thomas, pp. 18–46, 1943.

METHODS OF CONCENTRATING ACID-FAST BACILLI*

H. J. CORPER, M.D., AND CLARENCE R. NELSON, B.S.†

From the Research Department, National Jewish Hospital, Denver, Colorado, and University of Colorado School of Medicine, Denver, Colorado

This paper concerns an evaluation of various methods which have been proposed for concentration of tubercle bacilli in pathologic bodily fluids prior to their culture.

One of the earliest concentration methods was recommended by Uhlenhuth and co-workers[5, 18] who used "Antiformin", a trade preparation of uncertain composition consisting for the most part of sodium hypochlorite. This material digests the undesired tissue and cellular debris and does not disturb too seriously the morphology of acid-fast bacilli.

Aside from the use of the hypochlorite itself, there have been numerous attempts to use other agents as digestants, including the digestive enzymes, pyridine,[7] various concentrations of sodium hydroxide,[11] potassium hydroxide,[3] ammonia,[2] urea,[4] sodium carbonate (with autoclaving),[9, 10, 16, 19] and even distilled water[8] and acetic acid. Digestion is followed in some cases by precipitation or flocculation with aluminum, zinc or iron salts. Light benzene or gasoline products have been used after the hypochlorite digestion to float the bacilli in an intermediate surface layer. Likewise chloroform,[6, 12] which settles to the bottom, was used to obtain zonal separation. Commercial "Antiformin", however, unless tested frequently, is too uncertain in concentration to produce satisfactory digestion.

Various authors have claimed special merit and efficiency for their own particular modifications of the concentration technic; concentration of acid-fast bacilli in sputum samples have been recorded up to 240 times the original numbers.

In 1939, "Tergitol" (sodium octyl sulphate),[15] a new organic wetting agent, was introduced. It was claimed to have a more rapid and complete action than any other digestant used previously. However, it was soon modified. One part of "Tergitol penetrant 08", 1 part of 4 per cent sodium hydroxide and 1 part of water was used, but this was again replaced later by equal parts of "Tergitol 08" and Javelle water (contains sodium hypochlorite). The latter mixture gave a more uniform digestion but had to be prepared frequently. No controlled evidence was presented to confirm the value of "Tergitol" in sodium hypochlorite mixtures. More recently, "Clorox", a commercial alkaline sodium hypochlorite solution,

* This investigation was aided by a gift from Lothair S. Kohnstamm in memory of Madeline P. Kohnstamm.

Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 14, 1948.

Received for publication, October 5, 1948.

† The following participated in this work and its interpretations: Dr. M. L. Cohn, J. Shrewsbury, William Bain, Varnum Tefft, S. A. Hartman, Allison Sykes and David Minkowitz.

has been recommended[13, 14] and it was claimed that 15 per cent more positive results were obtained with it than by the smear method.

STANDARDIZATION OF DIGESTANT

It is obvious from the literature that commercial sodium hypochlorite is subject to deterioration and that it may lose over 50 per cent of its activity within one year.[17] Sodium hypochlorite can be tested for activity by a simple method which gives an accurate estimation both of the amount of sodium hypochlorite and of available chlorine.[1]

In routine examinations by concentration methods where definite quantities of hypochlorite solutions should be employed, standardization of these hypochlorite solutions becomes imperative.

Sodium hypochlorite can be prepared as follows: To 75 grams of pure sodium carbonate dissolved in 500 ml. of distilled water is added 50 grams of a stable high-test calcium hypochlorite.* The mixture is allowed to stand in the dark for 18 hours with occasional shaking. The precipitated calcium carbonate is removed by filtration, and the sodium hypochlorite filtrate is then stored in brown bottles at refrigerator temperature. Crystals may separate out at this temperature but can be redissolved by gentle warming. This solution is stable at refrigerator temperature and should retain its potency from three to six months. It should contain about 6 per cent of available chlorine.

EXPERIMENTS AND RESULTS

It is well known that tubercle bacilli present in naturally expectorated sputum have an extremely irregular distribution. In addition, there is the factor of dilution by saliva and other secretions from the upper respiratory tract. In spite of this, the direct smear method carefully performed has yielded fairly good results in most cases of "open" pulmonary tuberculosis. However, the method is limited by the small amount of material which can be examined microscopically in a reasonable length of time.

To note the effect of mechanical homogenization of a positive sputum upon the finding of bacilli, sputums were examined by smears before and after mechanical homogenization and before and after concentration with sodium hypochlorite.

It appears obvious from the results recorded in Table 1 that homogenization of positive sputums did not succeed in increasing the frequency of finding of bacilli either by the direct smear or by the sodium hypochlorite method. An attempt was then made to determine the value of "concentration" with a negative sputum (from a nontuberculous bronchiectatic patient) to which graded amounts of a fine suspension of tubercle bacilli had been added. The results of such a test, recorded in Table 2, indicate that a tenfold increase in bacillary concentration may be achieved. This finding should be considered in the light of results of culture methods in which a few bacilli can be expected to multiply and yield positive findings.

* H. T. H., manufactured by the Mathieson Alkali Works, Inc., New York, N. Y., is such a preparation.

TABLE 1

EFFECT OF MECHANICAL HOMOGENIZATION[a] OF SPUTUM ON THE DISTRIBUTION OF ACID-FAST BACILLI

SPUTUM NUMBER	METHOD OF EXAMINATION			
	Before Homogenization		After Homogenization	
	Direct smear[b]	NaOCl[c]	Direct smear	NaOCl
1	180[d]	3700	94	1140
2	0	27	0	62
3	8	29	0	584
4	6	1640	36	1272
5	34	5100	93	8000
6	0	73	5	31
7	0	172	14	600
8	55	1475	22	2000

[a] A motor driven mechanical paddle with pendulous motion, vibrating over a small arc at 1725 vibrations per minute, was used to homogenize the specimens.

[b] The direct smear was made by placing a uniform spread of suitable thickness for good examination, covering a width from $\frac{1}{2}$ to $\frac{2}{3}$ inch on a 3 x 1 inch slide.

[c] The NaOCl procedure was performed on 3 ml. of sputum using a standard technic with up to 3 volumes, depending on the consistency of the sputum, of a 3 per cent NaOCl solution. The material was mixed well for 10 to 20 minutes, centrifuged at 3000 r.p.m. for 20 minutes and drained. One drop of water was added to the sediment which was mixed well and all of it was then transferred to a slide, then air-dried, heat-fixed and stained for acid-fast bacilli.

[d] The numerals indicate the numbers of acid-fast bacilli found in 100 oil-immersion fields.

TABLE 2

EFFECT OF "CONCENTRATION" ON THE MICROSCOPIC DEMONSTRATION OF TUBERCLE BACILLI ADDED TO SPUTUM

METHOD	NUMBER OF BACILLI[a] ADDED TO 1 ML. OF MICROSCOPICALLY-NEGATIVE SPUTUM				
	10,000,000	1,000,000	100,000	10,000	1,000
Direct smear	214[b]	21	0	0	0
NaOCl	—	309	12[c]	0	0
NaOCl + "Tergitol 08" (Ref. 15)	—	390	6[d]	0	0

[a] 1 mg. of bacilli is equivalent to about one billion bacilli.

[b] The numerals indicate the average numbers of bacilli found per 100 oil-immersion fields.

[c] Of 19 separate determinations, 10 were negative. The number indicates the average count of bacilli found in 19 tests.

[d] 7 of 10 tests were negative.

In order to learn more about the effect of "Tergitol 08" with and without hypochlorite, both on recovery of tubercle bacilli and on its digestive action on sputum, the following tests were performed. In one set of tests, fine suspensions of tubercle bacilli were treated with saline solutions of different strengths, and

also with "Tergitol 08" and/or sodium hypochlorite. It was found that "Tergitol 08" compared with the isotonic saline solution, had no appreciable effect upon the tubercle bacilli as determined by the volume of the sediment of bacilli resulting after centrifugation. On the other hand, sodium hypochlorite alone caused a definite decrease in the volume of bacillary sediment, and this was not affected appreciably by the use of "Tergitol 08" in combination with the sodium hypochlorite. There was a definite tendency for "Tergitol 08" to clear the opalescence of the supernatant liquid after treatment with sodium hypochlorite. To a lesser extent, distilled water and hypertonic sodium chloride (6 per cent) solution also reduced the volume of bacillary sediment.

"Tergitol 08" exerts a clearing or gelatinizing action upon a positive tuberculous sputum, and this differs from that of the sodium hypochlorite which acts as a digestant of the sputum and results in a sediment of low volume and an opalescent supernatant fluid. The addition of "Tergitol 08" to the sputum does not enhance the digestive action of the sodium hypochlorite nor does it appear to favor its action in any perceivable manner so far as adding to its efficiency in enabling one to find acid-fast bacilli. It does, however, aid in clarifying the supernatant liquid but results in a slightly increased sediment. This increase is probably caused by gelatinization of the sputum which, however, does not appear to be of any practical value for increasing the efficiency of the method in detecting acid-fast bacilli.

An extensive study with sputums was also carried out in which two series of tests were performed, especially to compare the value of the concentration method with the direct smear method and to compare the straight sodium hypochlorite method with a combination of sodium hypochlorite and "Tergitol 08". It was found that "Tergitol" with sodium hypochlorite does not appreciably alter the positive findings with tuberculous sputum over those found with sodium hypochlorite alone. With "Tergitol" there is a slight difference in appearance in the final material but no alteration of the bacilli or their number in the sediment.*

SUMMARY

1. Concentration methods are less specific and less efficient than the biologic tests of culture or guinea pig inoculation.

2. The direct smear is a valuable procedure for detecting acid-fast bacilli when these are present in relatively large numbers in a specimen of sputum. This method of routine examination may also yield other valuable diagnostic information not to be gained by methods of concentration.

3. The concentration methods are of value only in those sputums containing relatively few tubercle bacilli.

4. The sodium hypochlorite method, originally suggested about 1908, appears to be as reliable for detecting the presence of acid-fast bacilli by concentration as any of the more complicated procedures involving precipitations, flotations or the addition of detergents.

* Editor's note: Because of lack of available space in the Journal, the detailed tabulations and experimental data have been omitted.

REFERENCES

1. Association of Official Agricultural Chemists: Official and Tentative Methods of Analsis. Ed. 6. Washington, D.C., p. 53, 1945.
2. Brauer, Kurt: Ein neues Verfahren zur Anreicherung von Tuberkelbazillen im Sputum. Deutsche med. Wchnschr., **44**: 266–267, 1918.
3. Ditthorn, F., and Schultz, W.: Die Anreichungsverfahren zum Nachweis von Tuberkelbazillen im Sputum. Centralbl. f. Bakt., I. Abt., Orig., **79**: 166, 1917.
4. Dold, H.: Ein neues Verfahren zur Homogenisierung von Sputum, Anreicherung und Reinzüchtung von Tuberkelbazillen. Beitr. z. Klin. d. Tuberk., **58**: 335–336, 1924.
5. Ellermann, V., and Erlandsen, A.: Nachweis von Tuberkelbazillen im Sputum. Ztschr. f. Hyg., **61**: 219–223, 1908.
6. Fourest, E.: Procédé simple et rapide de recherche des bacilles de Koch dans les expectorations, par homogenization et enrichissement. Compt. rend. Soc. de biol., **88**: 516–517, 1923.
7. Giraud, Marthe, and Debrien, E.: Recherche des bacilles tuberculeux dans les expectorants fluidifiés par la pyridine. Compt. rend Soc. de biol., **79**: 976–977, 1916.
8. Gorescu, C.: Nouveu procédé d'enrichissement des bacilles tuberculeux dans les crachats. Compt. rend. Soc. de biol., **86**: 889–890, 1922.
9. Greenfield, J. G., and Anderson, J.: Sedimentation of tubercle bacilli in sputum. Lancet, **197**: 423, 1919.
10. Hilgermann and Zitek: Konzentration der Tuberkelbazillen im Auswurf nebst gleichzeitiger Abtötung. Med. Klin. **16**: 959–960, 1920.
11. Koch, T.: Die Anreicherungsverfahren der Tuberkelbazillen im Sputum, nebst einem weiteren Beitrag. Centralbl. f. Bakt., I. Abt., Orig., **83**: 351–352, 1919.
12. Loeffler, F.: Ein neues Anreicherungsverfahren zum farberischen Nachweise sparlicher Tuberkelbazillen. Deutsche med. Wchnschr., **36**: 1987–1988, 1910.
13. Nagle, N., Lazarov, J., and Willet, J. C.: Use of sodium hypochlorite in the concentration of tubercle bacilli. Dis. of Chest, **10**: 47–53, 1944.
14. Oliver, J., and Reusser, T. R.: Rapid method for concentration of tubercle bacilli. Am. Rev. Tuberc., **45**: 450–452, 1942.
15. Petroff, S. A., and Schain, P.: New concentration and staining method for demonstrating acid-fast bacilli in sputa and other tuberculous matter. Quart. Bull., Sea View Hosp., **4**: 145–152, 1939. Sputum examination; additional data pertaining to digestion and staining. Quart. Bull., Sea View Hosp., **5**: 183–185, 1940.
16. Raphael, Theophile, and Eldridge, Nina: An improved concentration technic for the detection of tubercle bacilli in sputum. J.A.M.A., **75**: 245, 1920.
17. Thresh, J. C., Beale, J. F., and Suckling, E. V.: Examination of Water and Water Supplies. Ed. 5. Churchill: The Blakiston Co., pp. 635–636, 1943.
18. Uhlenhuth and Xylander: Antiformin, ein bakterienauflösenden Disinfektionsmittel. Arb. a. d. kaiser. Gesundheitsamte, **32**: 1–2, 1909.
19. Wooley, Stanley: A simple technic for concentrating sputum. J.A.M.A., **74**: 525, 1920.

STANDARD KLINE TEST OF CEREBROSPINAL FLUID FOR SYPHILIS

Employing Cardiolipin-Lecithin Antigen*

B. S. KLINE, M.D., and H. SUESSENGUTH, M.T. (ASCP)

From the Laboratory Department, Mt. Sinai Hospital, Cleveland, Ohio

The formula of the antigen emulsion for the spinal fluid slide test, previously described,[1] was found satisfactory with two different lots of standard cardiolipin and two different lots of standard lecithin. Subsequently, antigen mixtures of other lots of standard cardiolipin and of standard lecithin, although satisfactory for the preparation of the heated serum slide test emulsion were not satisfactory for the preparation of the spinal fluid slide test emulsion by the formula specified.

In a study of 13 different lots of standard cardiolipin and of 18 different lots of standard lecithin, antigen mixtures of different lots of cardiolipin and of one lot of lecithin gave similar results in the heated serum slide test, whereas mixtures of different lots of lecithin and of one lot of cardiolipin gave results of variable sensitivity.

The variable behavior in the slide tests for syphilis of different lots of standard lecithin has necessitated the titration of each lot with standard cardiolipin for the determination of the optimal ratio for use in the preparation of the slide test emulsions. This has been found to vary from 9 to 11 parts of lecithin to one part of cardiolipin. The variable potency of different standard lecithins has, furthermore, necessitated the determination of a more satisfactory formula for the preparation of the antigen emulsion for the spinal fluid slide test than that previously employed. Twelve new formulas with the specified amount of optimal antigen mixture of four different lots of cardiolipin, five different lots of lecithin, and different quantities of standard cholesterol, water and salt solution were tested.

The formula that gave the best results in the slide test of 850 spinal fluids, as judged by the results previously obtained with other tests, is described below.

With the new antigen emulsion, it was found that the results were better when the 1 per cent acetic acid was not used and when the test mixture was agitated for eight minutes instead of four minutes. It was also found advantageous to use 0.3 ml. of spinal fluid instead of 0.25 ml.

New Formula for Spinal Fluid Slide Test Emulsion

0.6 ml. 1 per cent Cholesterol Solution (Pfanstiel C.P. precipitated from alcohol for Kline tests)

0.4 ml. Distilled Water, pH 6 (Distillata†)

0.1 ml. Cardiolipin-Lecithin Antigen for Kline tests (LaMotte)

1.4 ml. 0.85 per cent Sodium Chloride Solution (Reagent, Merck)

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 14, 1948.

Received for publication, October 5, 1948.

† Obtainable from the Distillata Company, Cleveland, Ohio. This, or a distilled water of similar quality, is recommended for routine use.

Preparation of Emulsion for Standard Kline Test for Syphilis with Spinal Fluid

1. Into a round bottom 3 or 4 inch x 1 inch test tube, pipet the cholesterol solution.
2. Allow the distilled water in a pipet to run in rapidly by removing the finger of the left hand from the top and blowing in the last drop or two, at the same time vigorously rotating the tube on a flat surface with the right hand during the mixing and for ten seconds thereafter.
3. Add the cardiolipin-lecithin antigen from a 0.2 ml. pipet and rotate the tube vigorously on a flat surface for one minute.
4. Add the saline and rotate the tube with moderate vigor, on a flat surface for 30 seconds.
5. Centrifuge the emulsion for five minutes at 1100 r.p.m.
6. Decant the supernatant turbid fluid.
7. Add 0.6 ml. of saline to the sediment and rotate the tube vigorously for 30 seconds to distribute the particles completely and uniformly.
8. Transfer the emulsion to a 4 × ½ inch test tube.

It may require a little experimentation to determine the time and speed of the centrifugation to obtain a sediment with the optimum number of particles. The supernatant fluid should be somewhat turbid, not clear (see Fig. 1). The sediment should be in such an amount that when one drop (0.008 ml.) of the emulsion is added to 0.3 ml. of spinal fluid the mixture contains the optimum number of particles.

The emulsion, kept refrigerated, is satisfactory for use for at least 48 hours after preparation.

STANDARD KLINE TEST (CARDIOLIPIN-LECITHIN ANTIGEN) FOR SYPHILIS WITH SPINAL FLUID*

Spinal fluids, turbid with exudate, blood, or bacteria, or containing injected substances including horse serum, are unsatisfactory for testing.

Spinal fluids with slight turbidity or few particles, are centrifuged at high speed for 10 minutes, and the clear fluid is used for testing.

Technic

The present technic of the slide test for syphilis with spinal fluid differs little from that previously described by Kline and Rein[2] and is as follows:

1. Heat the spinal fluids in a water bath at 56 C. for five minutes immediately before testing them.
2. While the spinal fluids are being heated, place the required number of spinal fluid test slides in a holder (see Figs. 2, 3 and 4).
3. Into each chamber, pipet 0.3 ml. of the warm spinal fluid to be tested.
4. Into each chamber, drop one drop (0.008 ml.) of spinal fluid antigen emulsion.
5. Rotate the slides on a flat surface with moderate vigor for 30 seconds to distribute the emulsion and then for eight minutes move the holder gently but rapidly (about three complete movements a second) back and forth, a distance of one-quarter to one-half inch.

* Standard reagents and equipment for this test may be obtained from the LaMotte Chemical Products Company, Towson 4, Baltimore, Md.

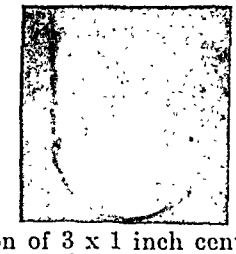


FIG. 1. Portion of 3 x 1 inch centrifuge tube with centrifuged emulsion for spinal fluid test showing sediment and turbid supernatant fluid.

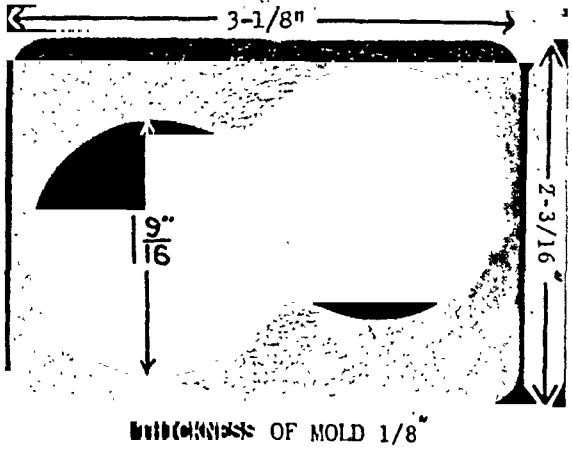


FIG. 2. Metal mold for preparing spinal fluid test slide.

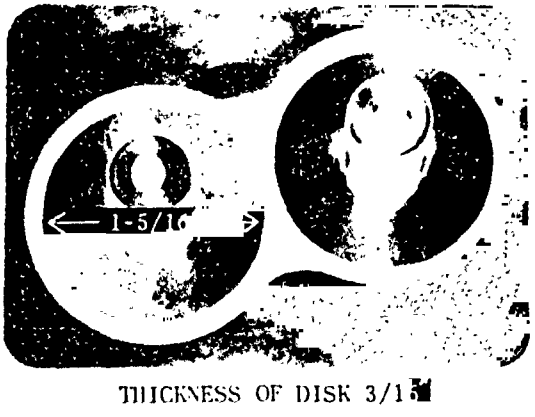


FIG. 3. Metal mold with metal disks in place forming wells to be filled with hot paraffin Vaseline mixture.

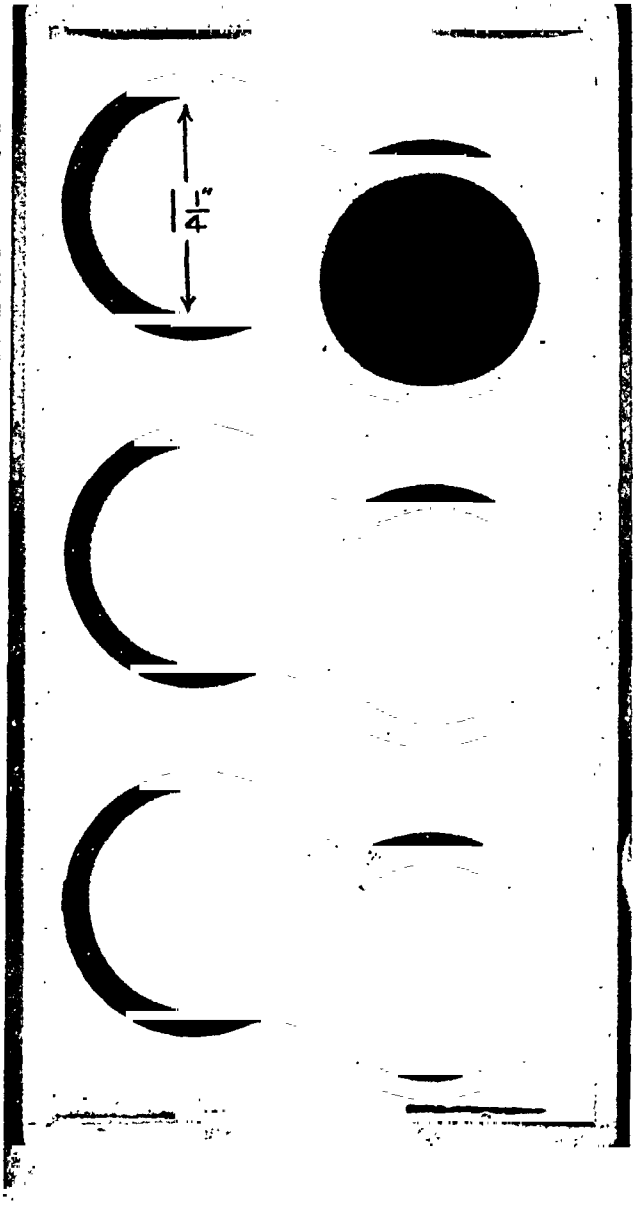


FIG. 4. Holder with 3 spinal fluid test slides in place.

6. The slides are examined at once through the microscope, at a magnification of 100 times, with the light cut down as for the study of urinary sediments. They are recorded in terms of 1 to 4 plus, according to the degree of clumping and the size of the clumps as for the serum test. For ease in reading the results, the slide is slightly tilted.

Titer and reagin unit determinations of syphilitic spinal fluids are performed as are those of syphilitic serums. The titer is the highest dilution giving a 2 plus or stronger reaction. The number of reagin units is the same as the dilution at which the last positive reaction is a 2 plus.

SUMMARY

Antigen mixtures of different lots of standard cardiolipin and of one lot of standard lecithin have given similar results in slide tests for syphilis. Antigen mixtures of different lots of standard lecithin and of one lot of standard cardiolipin, however, have given results of variable sensitivity.

Because of the variable behavior of different lots, it has been necessary to titrate each lot of lecithin with standard cardiolipin for the determination of the optimum ratio for use in the preparation of the slide test emulsions. It has furthermore necessitated the determination of a more satisfactory formula for the preparation of the antigen emulsion for the spinal fluid slide test than that previously employed. Twelve new formulas with the specified amount of antigen mixture of four different lots of cardiolipin, of five different lots of lecithin, and different quantities of cholesterol, water and salt solution were tested. The formula giving the best results in the slide test of 850 spinal fluids is described.

The present technic of the standard Kline test (cardiolipin-lecithin antigen) for syphilis with spinal fluid is detailed.

Acknowledgments. The spinal fluids were obtained from the Cleveland Clinic through the courtesy of Mr. Alfred Reich and from the Institute of Pathology, Western Reserve University, Cleveland, Ohio, through the courtesy of Mr. Karl Meinhardt.

REFERENCES

1. KLINE, B. S.: Cardiolipin antigen in the microscopic slide precipitation test for syphilis. Am J. Clin. Path., **16**: 68–80, 1946.
2. KLINE, B. S., AND REIN, C. R.: Microscopic slide precipitation test for syphilis with spinal fluid. J. Lab. and Clin. Med., **16**: 398–404, 1930.

A SPECIAL MICROSLIDE FOR CULTURE OF FUNGI*

M. L. LITTMAN, PH.D.

From the Department of Tropical Medicine and Public Health, Tulane University School of Medicine, New Orleans, Louisiana

During the past decade there has been an increasing awareness by the medical profession of the role of fungi as causative agents of certain cutaneous and systemic diseases. Nevertheless, culture diagnosis of fungous disease is still an uncommon procedure in many clinical laboratories. One reason for this has been the inadequacies of culture mediums for the primary isolation of pathogenic fungi; another has been the considerable skill and time required for preparation of slide cultures necessary for identification of the organisms; and finally a formidable obstacle has been the dearth of laboratory technicians trained in medical mycology. In many laboratories, therefore, no serious attempts are made either to isolate or to identify pathogenic fungi from clinical material.

Recently a new culture medium containing bile was described[6, 7] for the primary isolation of pathogenic fungi. This medium inhibits the growth of bacteria. It has proved to be highly successful in the culture diagnosis of cutaneous and systemic mycoses in human beings.[7, 8]

Johnson[3] has pointed out numerous difficulties encountered in the available slide culture technics[1, 2, 5] and the unsuitability of these methods for the rapid identification of pathogenic fungi. As an aid to our work a simple microslide was designed for the easy and rapid preparation of fungus microcultures, and it is hoped that this method will induce directors of many clinical laboratories to engage in culture diagnosis of fungus disease.

MATERIALS AND METHODS

Hanging drop slides with concave wells were found to be unsatisfactory. A microslide of ordinary thickness (1.5 mm.) was then constructed with a circular but *flat* well at one end of the slide and a frosted surface at the other end (Fig. 1).† The diameter of the well measured approximately 19 mm. and the depth was 0.61 mm. (0.024 inches). The well was shallow so that all planes of the chamber formed with a cover glass could be brought into focus with the low and high dry objectives of the microscope. The chamber was deep enough, however, to grow fungi satisfactorily. With parfocal low power and high power objectives it was possible to examine the preparation without cracking the cover glass, which frequently occurs in microcultures prepared by other technics.

Colonies of pathogenic fungi developing on Littman oxgall agar from clinical material are small, well separated and usually appear in almost pure culture on the plates. Sectors of 2 or 3 mm. of a suspicious colony are then transplanted to a Sabouraud agar slant and to the special microslide for culture. Numerous

* Received for publication, October 27, 1948.

† Available from Fisher Scientific Company, Pittsburgh, Pa.

varieties of saprophytic and pathogenic fungi have been grown in the special slide with satisfactory results and with considerable saving of time.

Slide culture preparations. The special slide, a No. 1 cover glass (18 mm. square) and a small square of absorbent cotton are placed within a petri dish (Fig. 1) and sterilized. Supports for the elevation of the slide are unnecessary. By means of a needle, the inoculum is placed in the center of the well (Fig. 1, Slide 2) but the slide itself is not removed from the petri dish. Four to six drops of sterile, melted potato dextrose agar[3] at a temperature of 45–50 C. are dropped

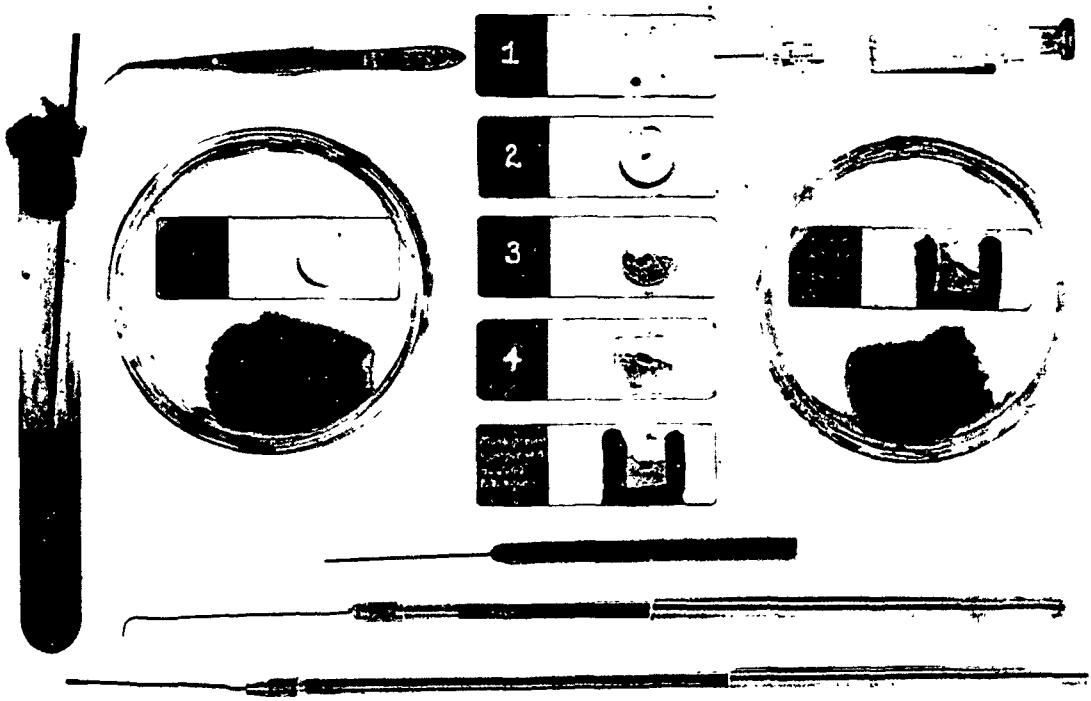


FIG. 1. Materials used in preparation of microculture of a fungus. Army Institute of Pathology, Acc. No. 218120-S1.

from a 2 ml. syringe into the *lower* half of the well so that the melted medium comes into contact with the inoculum but *does not* submerge it (Fig. 1, Slide 3). With a small pair of curved forceps previously flamed, the sterile cover glass is placed on the well, covering all of it except the upper periphery. The opening thus formed permits access of air into the microculture. The weight of the cover glass causes the melted agar to spread out bilaterally from the inoculum and forms an edge along which the fungus can grow (Fig. 1, Slide 4, and Fig. 2).

The desired temperature of agar is obtained by immersing the test tube containing the agar, in a beaker of water which is heated by a microburner under control of a thermometer. One 2 ml. syringeful of agar fitted with a 23-gauge $\frac{3}{4}''$ needle will fill from 20 to 30 slide chambers. If the needle becomes con-

taminated during the filling of the chambers it should be replaced by a sterile one. Sterile hot vaspar, consisting of 70 per cent hard paraffin and 30 per cent petrolatum by weight, is applied with a narrow swab to the sides and bottom edge of the cover glass but *not to the upper edge* (Fig. 2). The vaspar must be very hot in order to be spread smoothly and evenly.

It has been found more convenient in preparing many microcultures first to inoculate the special slides with all of the fungi under study. Then sterile, melted potato dextrose agar is added to one slide at a time, after which each chamber is immediately completed with a sterile cover glass. Not until all chambers are made are the cover glasses sealed with hot vaspar. This is done in one operation using one swab. The frosted surfaces of the slides are not labeled until after the chambers have been completed and sealed.

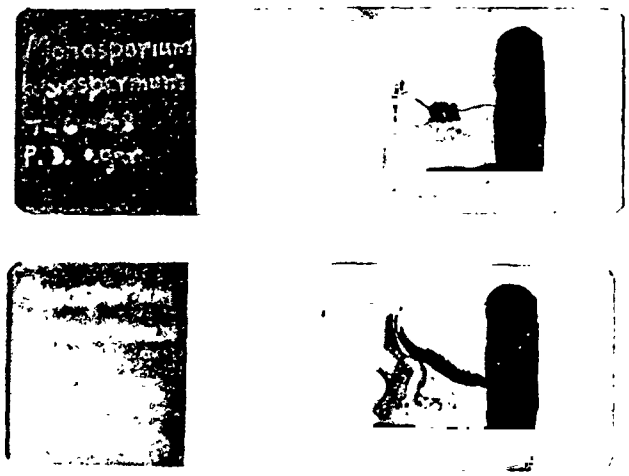


FIG. 2. Microculture preparation of a fungus before and after incubation. Note profuse growth along agar edge in lower slide. Army Institute of Pathology, Acc. No. 218120-S3.

By the foregoing procedure it has been possible to prepare as many as 30 mounts in one hour's time. Humidity is maintained by the addition of 5 ml. quantities of sterile distilled water or saline to the absorbent cotton. Incubation is provided either at room temperature in the shade or in an incubator at 30 C.

Once growth of a fungus has begun along the agar edge in the chamber, it seldom becomes contaminated with another mold. Following suitable incubation, the slide microculture may be removed from the petri dish, examined under the microscope with low power and dry high power objectives and returned to the dish for further incubation without danger of contamination. Because of the thickness of the chamber, it cannot be examined with the oil-immersion objective of the microscope unless one wishes to view only the upper plane. Such high magnification, however, is rarely necessary.

The identification of a pathogenic fungus rests on the colonial appearance of the organism on Littman's oxgall agar and Sabouraud's agar and on recognition

of the sporulating elements of the organism in microculture on potato dextrose agar. Pathogenic fungi can be isolated from clinical material and identified with certainty in from 6 to 14 days. The preparation of microcultures is unnecessary for numerous species of saphrophytic fungi which form characteristic colonies on culture mediums.

Sabouraud's dextrose agar is not employed as a culture medium in microcultures since it fails to promote the formation of spores by which a fungus may be identified. On the other hand, potato dextrose agar promotes rapid and abundant sporulation. According to Johnson, commercial preparations of this medium

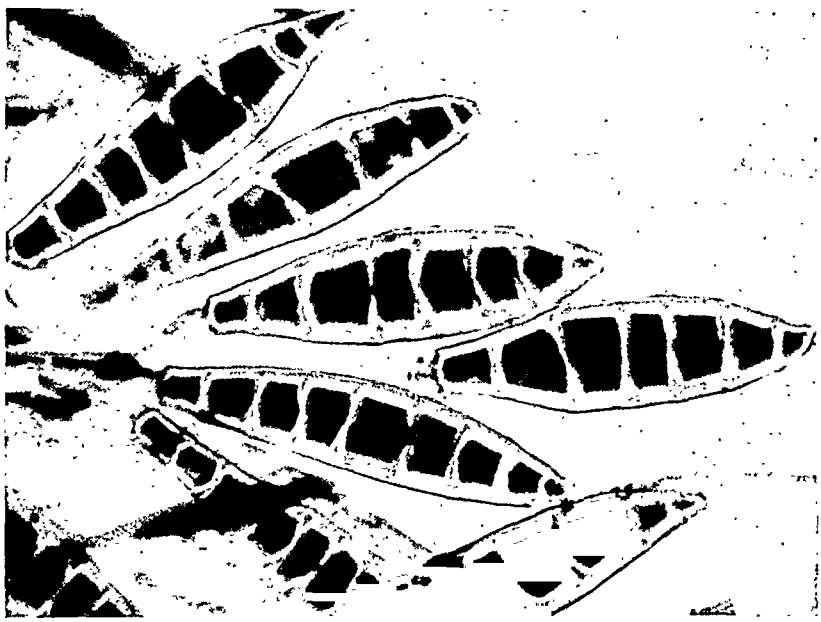


FIG. 3. *Microsporum canis.* Macroconidia developed in a microculture on potato dextrose agar. Cover glass mount with Lactophenol Cotton Blue stain. × 805. Army Institute of Pathology, Acc. No. 218120-285.

may be unsuitable for sporulation and he recommends that the medium be freshly prepared.* It should be pointed out that many different types of culture mediums can be used with the special slide.

Preparation of permanent mounts. An interesting behavior of a fungus growing inside a microculture is its formation of adherent growth along the inner surface of the cover glass. This is utilized for the preparation of permanent mounts. When the microculture has incubated for an adequate time, the vaspar seal is trimmed away with a razor blade. The cover glass of the microculture is pried away from the chamber carrying away a narrow flat line of mold growth which

* *Preparation of potato dextrose agar.*[3] Cut 250 grams whole potato into pieces the size of walnuts, steam in 500 ml. distilled water for 30 minutes. Pour through a wet towel, make up to volume, and add 1 per cent dextrose and 1.5 per cent agar. No pH adjustment is necessary. Heat to dissolve, tube and autoclave at 15 lb. for 20 minutes.

contains all of the diagnostic structures of the mold. The cover glass is permitted to fall on the tabletop, growth uppermost, on which is placed one small drop of lactophenol cotton blue.* The cover glass is then picked up by touching a clean ordinary glass slide to the drop of stain. Heat fixation of the mount is unnecessary. The slide is permitted to stand for 24 hours. Permount† or other synthetic resin diluted with its solvent to give it the desired fluidity is then streaked around the edges of the cover glass with a fine brush. The preparation is permitted to harden for 48 hours, after which it may be considered a permanent mount. A less destructible preparation may be made by covering the mount with a second larger cover glass (No. 1, 22 mm. square), again using Permount. The final preparation is allowed to harden for several days.

The result is a permanent, stained and stable preparation which carries identifying structures of the mold and which is not only indispensable for comparison with unknown organisms under examination, but is excellent for teaching purposes and perhaps for taking of photomicrographs (Fig. 3). After use, the special microslide is sterilized and washed and may then be used again.

The microslide has also been found useful in studying the morphology of members of the Actinomycetacea family, especially the genus Streptomyces. Here, as with molds, the organism grows along the inner surface of the cover glass. Permanent mounts, however, are made somewhat differently. The cover glass is fixed by heat and stained by Gram's method, after which it is mounted on a flat slide with either Permount, Canada balsam or Clarite. This preparation is suitable for examination with the oil immersion objective. For the rapid sporulation of Actinomycetaceae in slide culture a medium devised by Kelner and Morton[4] proved to be very satisfactory.

Sporulating medium for Actinomycetaceae. Potato extract is made by simmering 400 grams white potato slices for 40 minutes in 1000 ml. water (distilled water or, preferably, spring water). In the decanted liquid are dissolved 5 grams tryptone, 10 grams starch, 3 grams Difco beef extract, 2 grams glucose, 1.2 grams Na_2HPO_4, 0.8 grams KH_2PO_4, 0.1 gram $FeSO_4 \cdot 7H_2O$ and 15 grams agar.

SUMMARY

A special microslide for the culture of fungi is described, and its use for clinical laboratories discussed.

Acknowledgments are due to the Fisher Scientific Company for cooperation in the development of the special slide and to the Army Institute of Pathology, especially to J. Halsman and C. E. Edward, for the photographs.

REFERENCES

1. Brown, J. H.: A microculture slide for fungi. J. Bact., 43: 16, 1942.
2. Henrici, A. T.: Molds, Yeasts and Actinomycetes. New York: John Wiley and Sons, Inc., 296 pp., 1930.

* *Formula of Bactophenol Cotton Blue stain.* Phenol, crystals, 20 grams; lactic acid, syrup, 20 grams; glycerol, 40 grams; water, 20 ml. Dissolve with gentle warming, then add Poirrier's Blue (Cotton Blue, National Aniline Division, Allied Chemical & Dye Corporation, New York, N. Y.), 0.10 grams.

† Available from Fisher Scientific Company, Pittsburgh, Pa.

3. JOHNSON, E. A.: Improved slide culture technique for study and identification of pathogenic fungi. J. Bact., **51**: 689–694, 1946.
4. KELNER, A., AND MORTON, H. E.: Two antibiotics (Lavendulin and Actinorubin) produced by Actinomyces. J. Bact., **53**: 695–794, 1947.
5. LEWIS, G. M., AND HOPPER, M. E.: An Introduction to Medical Mycology. Ed. 2. Chicago: The Year Book Publishers, 342 pp., 1943.
6. LITTMAN, M. L.: A culture medium for the primary isolation of fungi. Science, **106**: 109–111, 1947.
7. LITTMAN, M. L.: Growth of pathogenic fungi on a new culture medium. Am. J. Clin. Path., **18**: 409–420, 1948.
8. LITTMAN, M. L., MCQUOWN, A. L., AND SCHNEIDAU, J. D.: Recent advances in the culture diagnosis of fungous disease. In press.

USE OF *XENOPUS LAEVIS* IN TESTING FOR PREGNANCY

Analysis of 1100 Tests*

MARLOWE DITTEBRANDT, M.D.†

For the past three years we have been using *Xenopus laevis* (South African claw-footed toad) in testing for pregnancy. Because the test has been considered by many to be in the experimental stage, we have kept complete records of all pregnancy tests performed and have made notes concerning the Xenopus and its peculiarities.

ANIMALS AND TANKS

The animals were mature females pretested to ascertain response to the pregnancy hormone. Up to 12 of these toads were placed in each 15 gallon fish aquarium. The water level in the tanks was maintained between 4 and 6 inches. As the animals are entirely aquatic, no rest-objects are needed in the tanks. Hardware mesh was used to cover the tanks. No attempt was made to regulate the temperature of the water, but the aquariums were kept in a room at about 70 F. The animals were obtained from Louis C. Herring, Orlando, Florida.

When changing the water in the tanks, the temperature of the clean water added was adjusted to that of the tank water. The toads were segregated according to whether they had given negative tests, positive tests, or were ready for use. Those animals giving negative tests were allowed to rest for at least one week; those giving positive tests, at least one month. The animals were fed ground horse meat twice a week. The tanks were cleaned thoroughly before each feeding and within twenty-four hours following each feeding.

Test tanks consisted of fish aquariums of 2-gallon capacity with hardware mesh tops, all tops being well weighted. The tanks were painted black on the outside on three sides and towels were thrown over the tops after the animals were placed in the tanks. This was done following the observation of Weisman and Coates[3] that exposure to sunlight or glare of the electric light inhibited or delayed oviposition.

METHODS OF CONCENTRATING URINE

1. *Acetone method.* The method of Weisman and Coates,[4] as modified by us, was used for approximately two years. It consists of taking 80 ml. of filtered urine, acidifying to litmus with glacial acetic acid and adding double the volume of acetone (160 ml.). After allowing the mixture to sediment for fifteen minutes, the entire quantity is filtered on a 60 ml. pyrex (F) glass sintered funnel under suction. The insoluble hormones and proteins are retained on the filter and are washed directly with two 20 ml. portions of acetone. The acetone is removed from the precipitate by suction. Two to 3 ml. of distilled water is added to the

* Received for publication, August 10, 1948.

† Address, 919 S.W. Taylor Street, Portland 5, Oregon.

precipitate which is emulsified by stirring with a glass rod. The liquid is filtered through a moistened filter paper and its pH adjusted to 5.5 (nitrazine paper) by the addition of 10 per cent sulfosalicylic acid. This solution is injected for the test.

2. *Kaolin method.* In 1940, Scott[2] published an original method for adsorbing the hormone from urine by means of kaolin. Zwarenstein and Duncan[5] modified Scott's method, and this procedure is the one we are now using. One hundred (100) ml. of urine are adjusted to a pH of 4.0 by adding 20 per cent hydrochloric acid with brom-cresol-green as indicator. The writer has found it more convenient to use a pH meter to make this adjustment. The kaolin is acid-washed according to the method of Hawk and Bergeim[1] and a 20 per cent suspension is made. Five ml. of well-shaken suspension is added to the urine and the mixture stirred thoroughly. After allowing the kaolin to settle, the supernatant urine is poured off and the remaining kaolin mixture centrifuged rapidly for about three minutes. The supernatant fluid is discarded. One drop of phenolphthalein indicator is added and sufficient N/10 sodium hydroxide to make alkaline. Generally, 3 ml. of alkali is sufficient. The mixture is stirred thoroughly and centrifuged rapidly for about three minutes. The supernatant is poured into small dishes and 20 per cent hydrochloric acid is added, drop by drop, until the pink color is just discharged; 2.5 ml. of this extract is injected at once into the toad. This final extract is sometimes turbid or forms a precipitate which in no way interferes with its use.

TECHNIC OF INJECTION

The material is injected subcutaneously through a 27 gauge needle, $\frac{3}{8}$ inches long, into the dorsal lymph space (Fig. 1). This space lies in the V-shaped area bounded on the sides by the linear markings and below by the cloacal folds. Our method of restraining these animals for injection is as follows: Cotton gloves are used and thoroughly moistened. A third glove is likewise dampened. An animal is grasped under the water in the tank and placed head first into the glove. The toad will quickly crawl to the finger end of the glove, and by clasping the cuff end closed, the animal will be held firmly. The cuff is turned back and the left leg and cloaca exposed, the right leg remaining covered by the cuff. By placing the fingers as shown (Fig. 2), the animal can move neither forward nor backward and the injection is easily made.

RESULTS

The results are divided into two groups, depending on the method of urine concentration. Errors in unpreserved, mailed specimens should not be charged against this procedure, for it is well known that the hormone producing the positive test rapidly disappears under such conditions.

1. *Acetone method.* In 533 tests, there were 16 false negative tests. Four of the 16 urines giving false negative results were unpreserved mailed specimens, which leaves a corrected accuracy of 97.8 per cent.

2. *Kaolin adsorption method.* In 567 tests, there were 10 false negative results.

Three of the 10 urines giving false negative results were unpreserved mailed specimens, which leaves a corrected accuracy of 98.8 per cent.

Analysis of 26 False Negative Results

7 urines were unpreserved mailed specimens (27 per cent).

9 urines were from patients who had missed a period for 14 days or less (34 per cent).

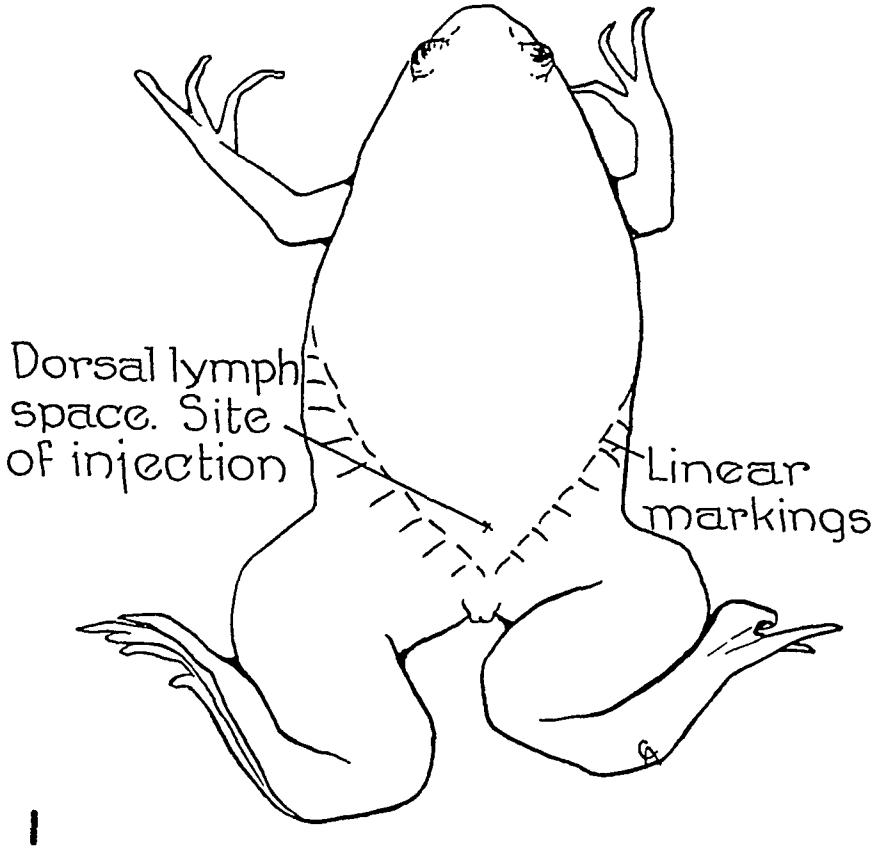


FIG. 1. *Xenopus laevis* showing linear markings and cloacal folds. Approximately ¾ natural size.

3 urines were from patients who had missed a period for 21 days or less (11 per cent).

2 urines were from patients whose periods were overdue 4 months or more (8 per cent)*.

Of the remaining 5 urines:

2 were from patients with ectopic pregnancy,

2 specimens contained one-half the required quantity of urine, and

1 specimen was accompanied by no information.

* Between the third and fourth months there is a rapid decrease in the concentration of gonadotrophic hormone. This hormone is the one to which the toad is most sensitive.

Ectopic Pregnancy

A total of 48 urines was tested in which the physician questioned the presence of an ectopic pregnancy. There were 13 correct positive tests and 28 correct negative. There were 2 false negative tests (95.4 per cent accuracy).

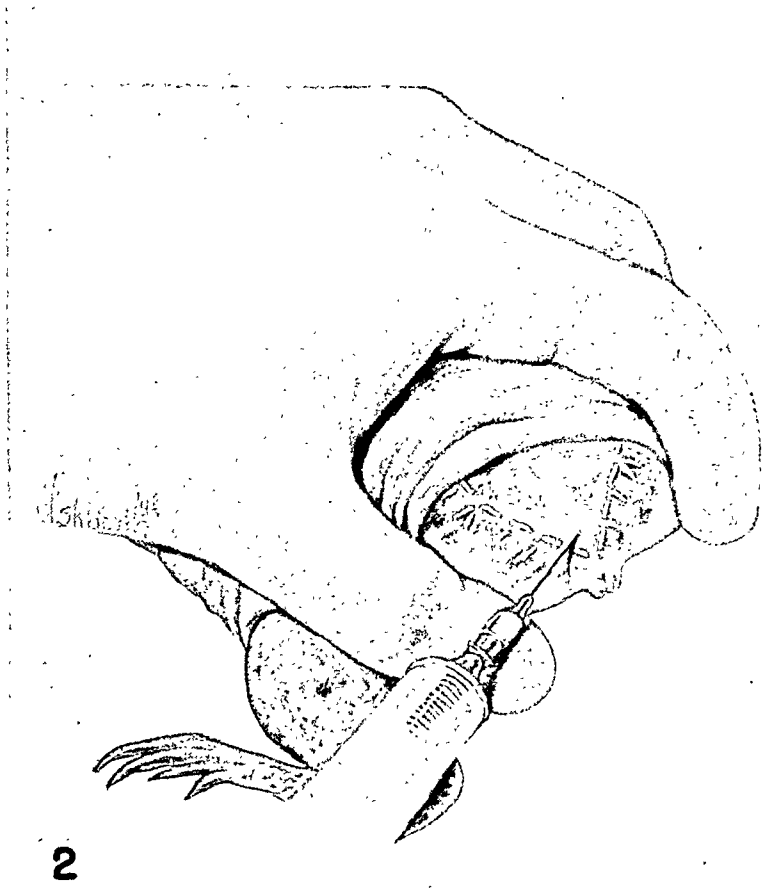


FIG. 2. Drawing from actual demonstration showing author's method of restraining animal for injection.

Hydatid Mole

In 8 urines tested there was a question of hydatid mole. The quantitative method was used. There were 6 correct negatives and 2 correct positives (100 per cent accuracy).

ANIMAL MORTALITY AND DISEASES

Of a total of 103 toads, we have lost 28. Causes of death were as follows:

1. Escaped from tanks, 3. The top of the tanks should be heavily weighted, especially the small test tanks. These animals cannot survive out of water.

2. Skin infection, 4. Occasionally ulceration and local inflammation will occur

at the site of injection. Prompt treatment is needed to save the animal and consists of isolation of the toad in a cool place in a tank containing from 10 to 15 drops saturated aqueous methylene blue per gallon, immersion for one hour daily in 1 to 10,000 permanganate, rinsing in clear water and replacing in methylene blue.

3. Intussusception, 2. Two animals that died showed at autopsy intussesception of the duodenal end of the small gut into the stomach. If the toad is not used for at least twenty-four hours after feeding, this complication probably will not arise.

4. Caught under wire, 2. When we were employing mesh bottoms in the test tanks, two animals dislodged these sufficiently to become wedged underneath.

5. Respiratory paralysis, 11. This complication accounts for the greatest loss in animals. It was commonly encountered in employing the acetone precipitation method. Since the kaolin adsorption method has been used, there have been no respiratory reactions.

6. Paralysis of hind legs, 4. Within the past six months the writer has noted severe and prolonged contractions in the hind leg muscles of some of the animals. In 4 cases it was sufficiently severe to interfere with the ability of the animal to come to the surface for air. These animals died. After consultation with the seller, it was decided that this muscular irritability might be due to thiamin deficiency. Approximately 10 mg. of thiamin have been placed in each large tank two or three times per week, and this complication has not been observed since.

SUMMARY

The experience we have had with the use of *Xenopus laevis* in testing for evidence of pregnancy is most satisfactory. The toads are ideal laboratory animals, requiring a minimum amount of space and care.

REFERENCES

1. Hawk, P. B., and Bergeim, Olaf: Practical Physiological Chemistry. Ed. 10. Philadelphia: The Blakiston Co., p. 253, 1931.
2. Scott, L. D.: Concentration and detoxification of human urine for biological pregnancy diagnosis. Brit. J. Exper. Path., **21**: 320–324, 1940.
3. Weisman, A. I., and Coates, C. W.: Effect of illumination on egg-extrusion reaction of *Xenopus laevis* in the frog test for pregnancy. Endocrinology, **35**: 68–69, 1944.
4. Weisman, A. I., and Coates, C. W.: The South African Frog (*Xenopus laevis*) in Pregnancy Diagnosis. New York: New York Biologic Research Foundation, 134 pp., 1944.
5. Zwarenstein, H., and Duncan, D. G.: Ten years of the Xenopus pregnancy test. Clin. Proc., **3**: 186–193, 1944.

A DIVIDED CULTURE PLATE*

JOSEPH FELSEN, M.D.

From the Laboratories of the Bronx Hospital, New York, New York

The divided culture plate described in this communication was devised primarily for use in isolating intestinal pathogens in cases of infectious diarrhea. The plate consists essentially of an ordinary 94 mm. Petri dish bottom which is separated into quarters by two smooth bisecting ridges of glass 4 mm. in height. All edges and corners are rounded for easy cleaning. The inside height of the

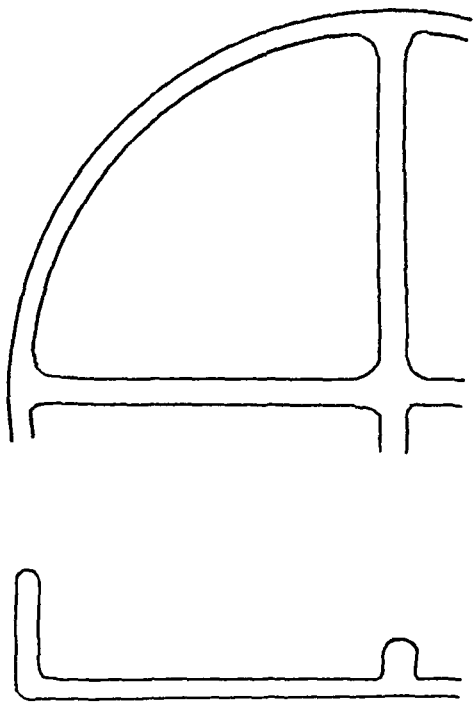


FIG. 1. Actual size of one cell (1/4 plate) of divided culture dish, top and side views.

bottom is 13 to 15 mm. and the covers are identical with those of ordinary Petri dishes.

The plate accomodates four differential mediums, such as Endo's, S S, desoxycholate citrate agar and MacConkey's or EMB. Each is poured into its own quarter-cell to a height of approximately 3 mm. This is 1 mm. below the top of the dividing ridge. The mediums are then seeded in the usual manner by loop, swab or spray, with or without preliminary enrichment. We have found

The Divided Culture plate (No. 23003-S2) is available from the Kimble Glass Co., Toledo, Ohio.

* Received for publication, November 24, 1948.

it expedient to use the sigmoidoscopic crypt aspiration-spray culture method since almost all of our cultures are taken directly from the intestinal mucosa by direct aspiration of the exudate into a heavy walled, glass capillary tube.[1] A few drops are drawn up into the distal bulbous portion by means of a rubber bulb. Part of the exudate is then blown on the culture medium from a height of 5 to 10 cm. The remainder is expressed on a slide for wet smear examination. A similar procedure may be followed with stools by first diluting the latter and aspirating, then expressing the suspension into the fecal container. Enough suspension adheres to the wall of the capillary tube to assure discrete colonies after spraying. There is also available a rectal culture tube[2] not requiring the use of a sigmoidoscope. The exudate is aspirated into a small concavity of a metal tip which is then sealed by a half turn of the outer metal tube. It may be cultured immediately or the material may be transported in its little sealed chamber.

Many other uses may be found for the divided culture plate, such as in antibiotic assays, blood titer determinations or bacteriophage studies. The economy of labor, space and mediums, as well as convenience in studying colonial reactions in differential mediums, makes the divided culture plate particularly useful in the isolation of intestinal pathogens.

REFERENCES

1. Felsen, J.: Crypt aspiration; spray culture method for the isolation of B. dysenteriae. J. Lab. and Clin. Med., **23**: 630-632, 1938.
2. Felsen, J.: New rectal culture tube. Science, **106**: 275, 1947.

DIOXANE METHOD OF DEHYDRATION OF TISSUES FOR PARAFFIN EMBEDDING*

THOS. F. WALKER, M.D.

From The Walker Laboratories, Great Falls, Montana

After having used the frozen section method for routine examination of tissues for thirty years, we have changed to the paraffin method, largely because of the simplification of this method made possible by the use of dioxane. The only

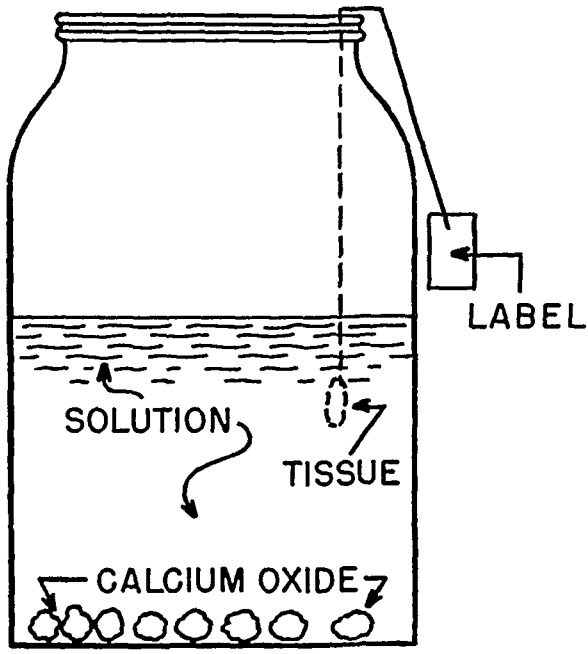


FIG. 1. Diagram indicating method of suspending tissue in dioxane.

procedure in the use of dioxane which, we believe, is original with us, is the method of securing rapid penetration of the tissue by dioxane without agitation of the tissue. This is accomplished by suspending the tissue in dioxane by means of a thread. The water in the tissue is heavier than the dioxane and as it diffuses out of the tissue, sinks to the bottom of the solution where it is absorbed by calcium oxide. Our procedure is as follows:

1. After fixation in formalin, small pieces of tissue, not over 5 mm. in thickness, are strung upon a piece of thread using an ordinary sewing needle. Curettings and small pieces of tissue may be placed within a piece of surgical gauze, the edges of which are gathered together to form a small bag which is suspended in solution by a thread. Air trapped in the bag which may interfere with penetration of the tissues by dioxane, may be forced out by applying gentle pressure on the submerged bag with a pair of forceps. As

* Received for publication, October 12, 1948.

many as 6 or 8 pieces of tissue may be strung upon the same thread. To the opposite end of the thread suspended on the outside of the container (Fig. 1) is attached a small paper label bearing the number of the tissue.

2. The threaded tissue is suspended in dioxane solution (dioxane 3 parts, water 1 part) contained in a one-quart glass, screw-cap "fruit jar". The thread is drawn over the edge of the jar so that the tissue is suspended just below the surface of the solution, the end of the thread to which the label is attached being held in place on the outside of the jar by a strip of adhesive plaster. Usually securing of the thread on the outside of the jar is unnecessary since the buoyancy of the solution, plus the weight of the thread and label, is sufficient to suspend the tissue at the proper level. The lid is pressed firmly upon the mouth of the jar. When no tissues are suspended in the solution, the lid is screwed on to prevent evaporation.

3. At the end of one hour, the tissues are removed from the solution and suspended in a jar of undiluted dioxane. Here they remain for from 3 to 48 hours, according to convenience. The bottom of the jar is covered with purified lump calcium oxide to a depth of 2 or 3 cm. (to absorb water).

4. From the undiluted dioxane the tissues are transferred to and suspended in melted Tissuemat* (equal parts having melting points 52–54 C. and 54–56 C.) or paraffin in an open fruit jar in a constant temperature water bath. The threads are attached to the inside top edge of the jar by pressing them firmly against a ring of paraffin adherent to the jar. If the tissues can be left in melted Tissuemat for 8 to 15 hours, it is unnecessary to use more than one change of Tissuemat.

If a more rapid method is desired, the tissue (not over 3 mm. thick) may be passed through three changes of Tissuemat, being left in the first change 15 minutes, in the second 45 minutes and in the third from 1 to 2 hours. The jars of Tissuemat are left in the heated water bath uncovered. The dioxane transferred with the tissues evaporates so that the only attention necessary is to replenish the Tissuemat occasionally.

5. When the tissues are to be mounted, they are transferred from the Tissuemat to the mold. The thread is cut with a pair of warm scissors and the tissue oriented in the mold with warmed forceps.

The advantages of this method are simplicity and economy of time, space and expense. The only equipment necessary is a half dozen screw-cap quart fruit jars and a constant temperature water bath. These occupy little space in the laboratory.

It is necessary to make but two transfers after the tissues are started: from the dioxane solution to the undiluted dioxane, and from the undiluted dioxane to the Tissuemat. The dioxane solution becomes discolored after a time and a layer of liquid fat accumulates on the surface. It should be filtered from time to time. The undiluted dioxane should be filtered from time to time and fresh calcium oxide should be added. We have run through as many as 300 tissues without renewing the dioxane.

* Product of Eimer and Amend, New York, N. Y.

AN IMPROVED SIMPLIFIED TECHNIC FOR COLLECTING MULTIPLE BLOOD SPECIMENS*

CHARLES A. HUNTER, PH.D., AND FRANK VICTOR, B.S.

From the Division of Public Health Laboratories, Kansas State Board of Health, Topeka, Kansas

For a number of years, the Division of Public Health Laboratories, Kansas State Board of Health, has been conducting evaluation studies on the performance of serologic tests for syphilis, which required the collection of multiple

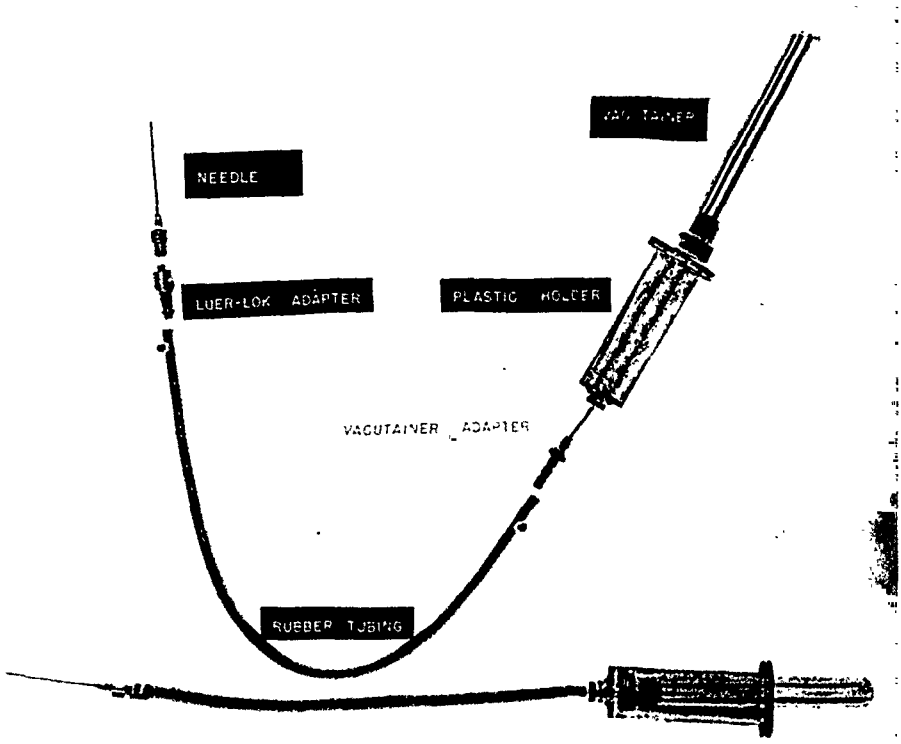


FIG. 1. Showing the separate parts and the assembled outfit.

specimens of blood for each donor. This was accomplished by using 20 ml. syringes and a needle with a large bore. The method necessitated the transfer of syringes, with some loss of blood, which was somewhat frightening to the donor. Also, during switching of syringes there was some discomfort to the donor. The number of syringes used per day was approximately 200. The cleaning and matching of this number of syringes was no simple task, due largely to the fact the location of the identifying number was improperly placed by the

* Received for publication, October 25, 1948.

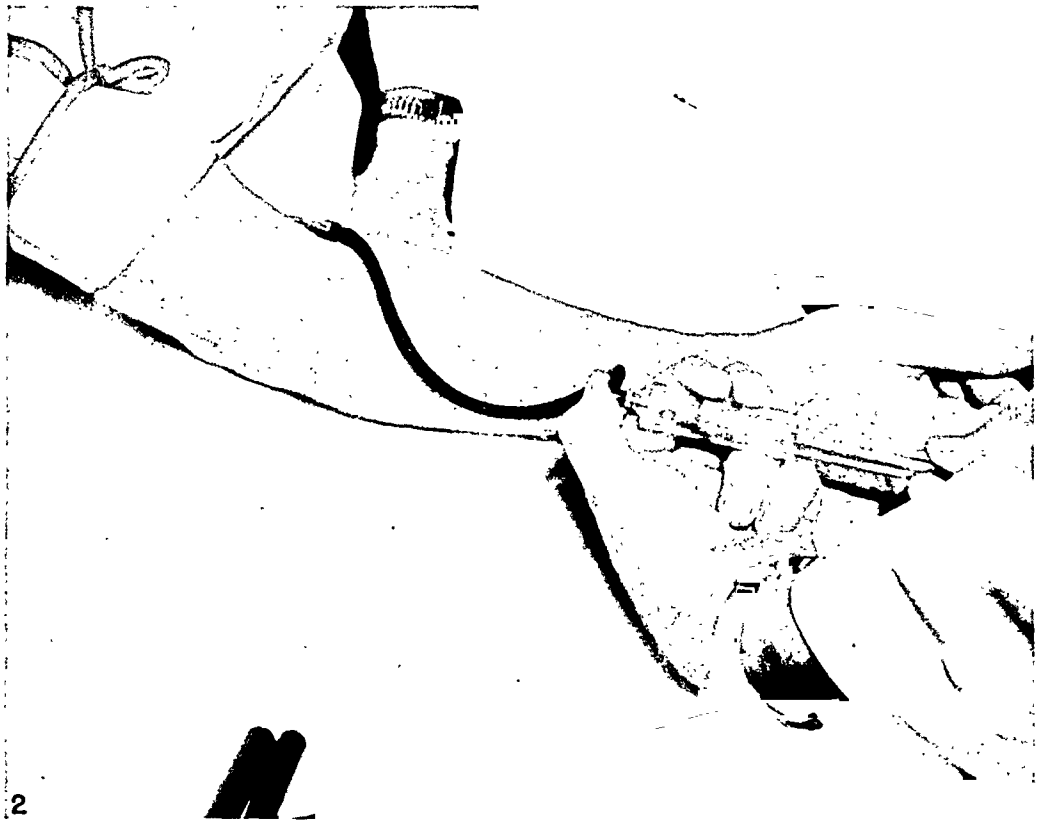


FIG. 2. Showing method of stopping flow of blood.

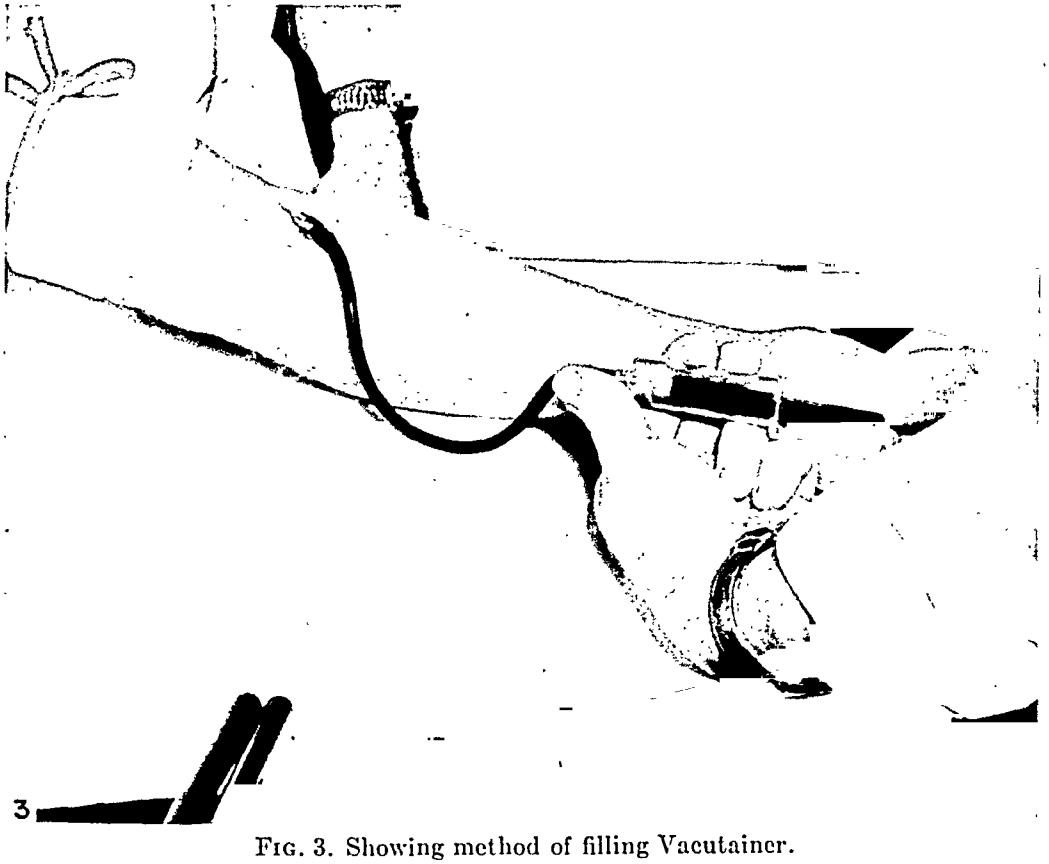


FIG. 3. Showing method of filling Vacutainer.

manufacturer. Also, the expense of the original equipment and the loss through breakage were high.

In the fall of 1947, Becton, Dickinson & Co. (Rutherford, New Jersey), suggested the use of Vacutainers to eliminate the trouble we have previously encountered. Their representative, Mr. Joseph Kleiner, came to Kansas and spent some time working with us in developing a more practical method.

The improved outfit consists of a gum amber tube approximately 8 inches long and 5 mm. in diameter, with a Luer-Lok needle adapter attached to one end, and to the other end the Vacutainer adapter, which screws into the plastic shield (Fig. 1). Our experience showed that the original adapter and needle were not satisfactory and Becton, Dickinson & Company made several different types of adapters which were tested. The adapter which proved most satisfactory has an 18-gauge needle and ferruled shank ($\frac{5}{8}$ inch in length). The increased length of the shank makes it easier to cut off the flow of blood during transfer of Vacutainers. The tube and adapter are sterilized in towels and the needles in constricted test tubes.

In the collection of blood, the size of needle to use is determined upon observation of the donor; generally 18- or 19-gauge needles are used. The sterile needle is attached to the Luer-Lok on the rubber tube and inserted in the vein. The blood flows down the tube and is stopped by placing the thumb over the end of the adapter (Fig. 2). The Vacutainer is then inserted in the shield and the blood collected (Fig. 3). During transfer of Vacutainers, the flow of blood is stopped, as mentioned before, by placing the thumb over the end of the adapter. It was found that 50 tubes of blood could be collected in about 4 minutes. This method of collection not only is fast but it is also quite inexpensive as compared to the older method. The only cleaning required includes that of the rubber tubing, connections and needle. The use of Vacutainers not only reduces the time of bleeding, but also eliminates the corking of many hundreds of tubes of blood per day. In fact, when Vacutainers are used there is no further work involved except sorting the tubes for mailing.

Previously, when syringes were used, the process required about six times as much time as is required in using Vacutainers.

SUMMARY

1. The use of Vacutainers, as described, for collecting multiple blood specimens provides a simple, rapid, and economical procedure.

2. The new adapter facilitates the transfer of Vacutainers without loss of blood.

USE OF SILICONE-TREATED TEST TUBES TO OBTAIN SERUM FROM BLOOD*

RALPH D. TURNER, MARGARET C. LAWLESS, ELLEN CLEARY, AND ELIZABETH M. FRENCH,

From the Rochester Regional Blood Program, Rochester, New York

The following measure will provide a good yield of clear serum in specimens of blood routinely collected for serologic tests.

Using a six-inch, cotton tipped, wooden applicator, the interior of the test tubes (100 x 15 mm.) is wiped with G-E Dri-Film (silicone).† After the Dri-Film has dried, the tubes are rinsed thoroughly with distilled water in order to remove the slight trace of hydrochloric acid in the tubes. When the tubes are dry they are sterilized, preferably in the autoclave, since excessive heat may injure the Dri-Film.

It was found that the blood specimens in silicone-treated tubes showed greater clot retraction, thus providing a greater yield of clear serum, and that they were much less subject to hemolysis despite considerable agitation.

* Received for publication, December 14, 1948.

† Available from The General Electric Company, Chemical Department, Pittsfield, Massachusetts.

PREPARATION OF FOLIN-CIOCALTEU'S PHENOL REAGENT

USING 30 PER CENT HYDROGEN PEROXIDE*

DAVID O. ROSBASH, PH.D.

From the Laboratory of the Veterans Administration Hospital, Roanoke, Virginia

One of the difficulties encountered in preparing the Folin-Ciocalteu reagent[1, 2] in small laboratories or in laboratories possessing inadequate facilities for removal of fumes is the necessity of using bromine to oxidize the complex phosphotungstomolybdic acid and produce the characteristic bright yellow color of this reagent. Bromine is a dangerous reagent to handle, being quite irritating and toxic. The storage of this reagent also presents a problem for most clinical laboratories.

It has been found that 30 per cent hydrogen peroxide may be used to replace bromine as an oxidizing agent for preparing the phenol reagent without resorting to the use of fume hoods or other special precautions. Small amounts of the hydrogen peroxide are added to a boiling solution of the phenol reagent until the proper shade of yellow is reached; an excess causes no harm to the phenol reagent and it is easily boiled away. The reagent prepared in this manner, when tested against the reagent prepared with bromine, showed no differences in either the determination of protein by the Greenburg method or in the determination of acid or alkaline phosphatases by the King-Armstrong method.

When the phenol reagent has become discolored by contamination, it is easily regenerated to its original quality by treatment with the 30 per cent hydrogen peroxide solution.

REFERENCES

1. HAWK, P. B., AND BERGEIM, O.: Practical Physiological Chemistry. Ed. 11. Philadelphia: The Blakiston Company, p. 755, 1937.
2. TODD, J. C., AND SANFORD, A. W.: Clinical Diagnosis by Laboratory Methods. Ed. 10. Philadelphia: W. B. Saunders Company, p. 412, 1943.

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author.

Received for publication, September 1, 1948.

A CONVENIENT SUPPORT FOR SEDIMENTATION TUBES*

E. M. ABRAHAMSON, M.D.

From the Jewish Hospital, Brooklyn, New York

This report describes a newly designed support† for sedimentation tubes (Fig. 1) that has certain advantages.

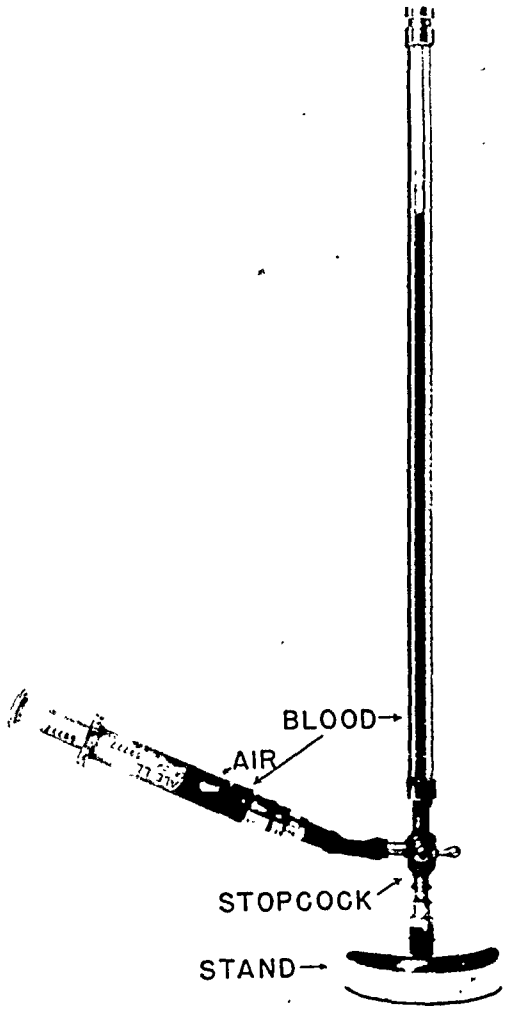


FIG. 1

1. If the apparatus is placed on any flat surface it will hold the tube in a vertical position. This is especially useful if a sedimentation test is performed in the patient's home.

* Received for publication, November 29, 1948.

† Made by Becton, Dickinson & Company, Rutherford, N. J.

2. The blood and anticoagulant are mixed without transfer in the syringe that is used for obtaining the sample.

3. The tube is filled from the bottom by positive pressure, thus avoiding the introduction of air bubbles which frequently get into the tube when it is filled by aspiration.

4. It is easy to introduce blood up to the level of the zero mark.

5. Repeated observations can be made on the same sample.

6. The entire apparatus is readily cleaned by rinsing under the tap.

The operation is very simple. One secures a little less than 2 ml. of blood by venipuncture. The plunger expels all but 1.8 ml. of blood from the barrel of the syringe. The tip of the needle is immediately dipped into a 3 per cent solution of sodium citrate which is drawn into the syringe until the total volume is 2.0 ml. The needle is removed and about 0.5 ml. of air is drawn into the syringe. The contents are thoroughly mixed by repeated inversions of the syringe which is then attached to the apparatus as shown in Figure 1, the syringe being elevated to keep the air from the tip. The stopcock is turned so that the syringe and tube are connected. The citrated blood is expelled from the syringe into the tube. When the level reaches the zero mark, the stopcock is closed and the apparatus is allowed to stand for an hour, with the syringe resting on the table, at the end of which time the sedimentation rate is read. If, for any reason, it becomes necessary to check the reading, the stopcock is opened and the blood is drawn from the tube into the syringe. The syringe is disconnected, its contents are mixed again and the apparatus is reassembled for a second determination.

NEWS AND NOTICES

An Appreciation to Dr. Kano Ikeda For Years of Service on the Board of Registry

The following communication has been received from Dr. Lall G. Montgomery, Chairman of the Board of Registry of the American Society of Clinical Pathologists:

"After having served continuously on the Board of Registry since its establishment in 1928, Dr. Kano Ikeda has now retired from membership on this Board. He was a member of the original committee appointed to study the problems of training and registration of laboratory technicians. Following the death of Dr. Ward Burdick, Dr. Ikeda carried on as chairman, and when the Board of Registry was established as a standing committee of the American Society of Clinical Pathologists, Dr. Ikeda was made a member of the Board, of which Dr. Philip Hillkowitz was the chairman.

"The Board of Registry wishes to take this opportunity to express its appreciation of the many years of unselfish service that Dr. Ikeda has given. No other person knows as much as he concerning the training and certification of medical technologists. His deep interest in the problems of medical technologists and the Board, his unbiased consideration of these problems and his forward-looking views, have made his services invaluable not only to the Board of Registry, but to the practice of medical technology as a whole.

"We know that Dr. Ikeda will continue to have a sincere interest in the affairs of the Registry and in the elevation of medical technology as a profession."

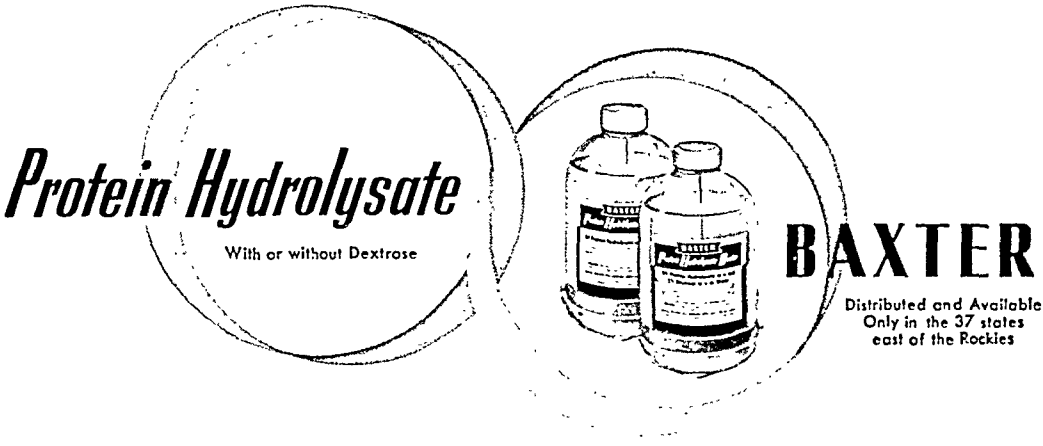
The Canadian Society of Laboratory Technologists

The Canadian Society of Laboratory Technologists will hold its 1949 convention in the Chateau Laurier, Ottawa, Ontario, Canada, May 20 and 21, 1949. Further information may be obtained by writing to Mr. J. R. Wilson, Ottawa Civic Hospital, Ottawa, Ontario, Canada.



Bovine blood can be collected and handled under carefully controlled, sanitary conditions. Protein Hydrolysate, Baxter, is prepared from the plasma of this blood because plasma proteins properly processed are good proteins*. Enzymatically digested, the proteins are converted into amino acids and peptides with a minimum change in structure. A new booklet, Protein Hydrolysate, Baxter, is yours for the asking. Baxter Laboratories, Morton Grove, Illinois.

Journal of the American Dietetic Assn. Vol. 23 #10 Page 841 October 1947.



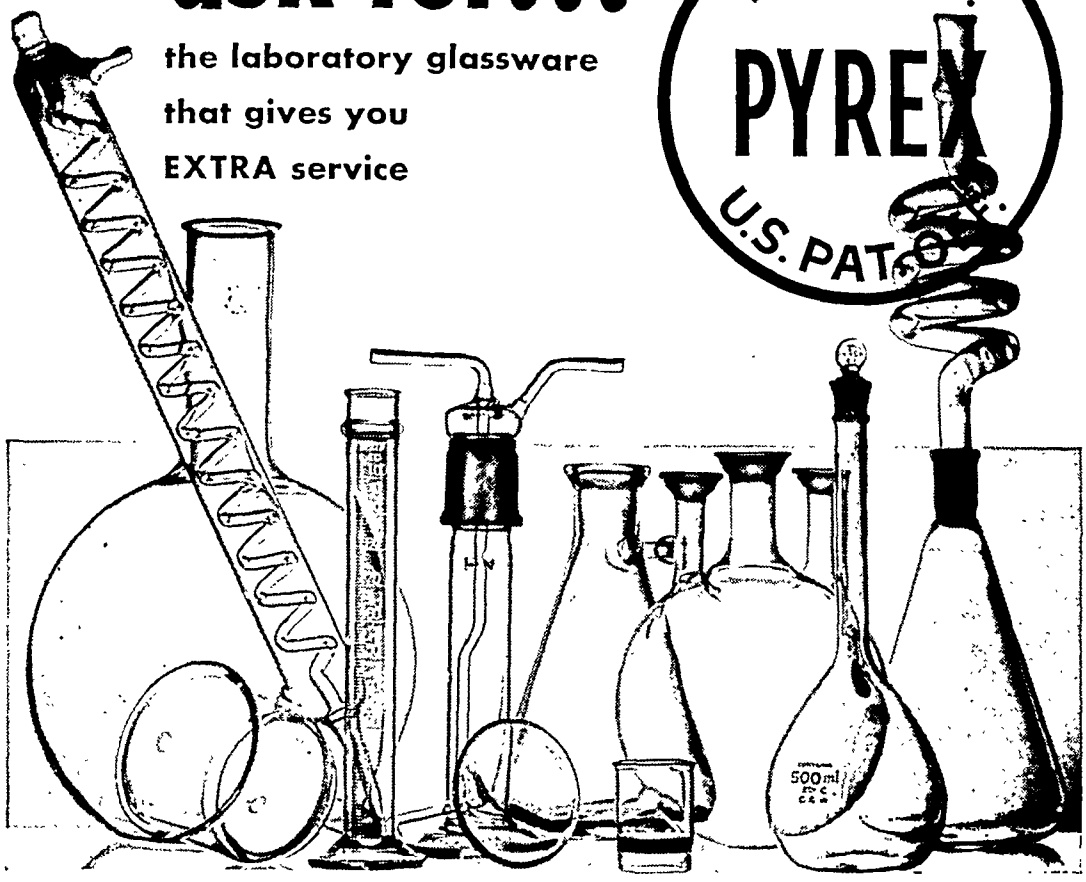
American Hospital Supply Corporation • General Offices, Evanston, Illinois

(In writing to advertisers, please mention the Journal—it helps.)

ask for...

the laboratory glassware that gives you EXTRA service

T.M. REG. PYREX U.S. PAT. OFF.



Look into your laboratory glassware instead of through it and you will specify PYREX brand, made of Corning's balanced glass No. 774!

This is the glass in which physical and chemical characteristics, so essential to accuracy, durability and economy, are carefully balanced to give you extra service.

This is the glass that is blown and fabricated by experts long skilled in the art of handling low expansion glasses. These men give every item the personal touch of careful workmanship. Quality is controlled from raw materials to finished product.

In short, Corning makes sure that everything is done right to give you the best possible product at the lowest possible cost. Ask *your* laboratory dealer for PYREX brand . . . you'll save money!

CORNING means research in GLASS

CORNING GLASS WORKS, CORNING, N.Y.

LABORATORY GLASSWARE

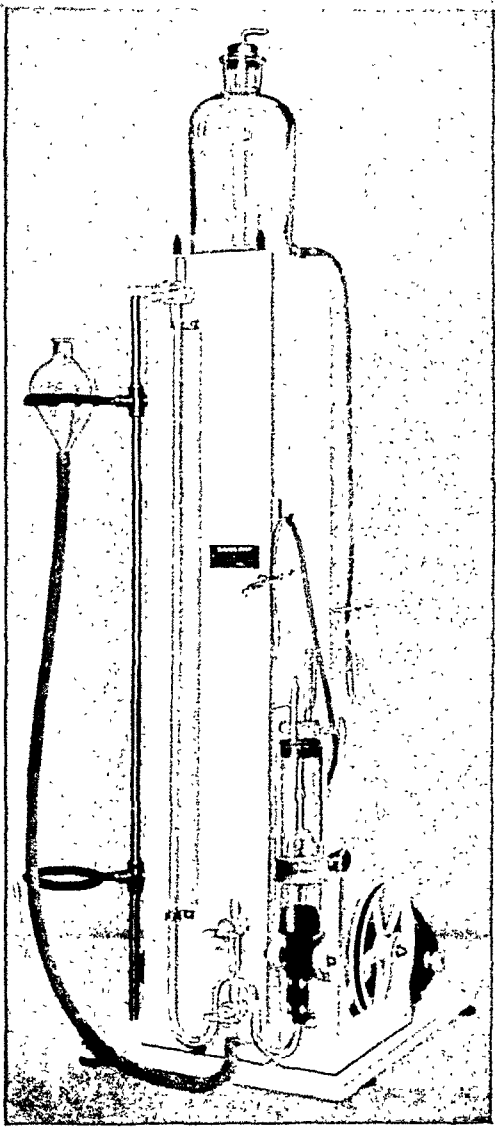
Stocked by Leading Laboratory Supply Houses

TECHNICAL PRODUCTS DIVISION: LABORATORY GLASSWARE • GLASS COMPONENTS
GLASS PIPE • GAUGE GLASSES • LIGHTINGWARE • SIGNALWARE • OPTICAL GLASS

(In writing to advertisers, please mention the Journal—it helps.)

BLOOD GAS APPARATUS
(Manometric)
VAN SLYKE-NEILL

- ALL METAL CONSTRUCTION
- VIBRATION FREE
- SILENT OPERATION
- LONG SERVICE LIFE
- ALL CALIBRATION MARKS PLAINLY VISIBLE



The welded steel tower contains a non-glare fluorescent tube to illuminate the manometer. All calibration marks on the manometer and the extraction chamber are plainly visible. The base, carriage, pulley and carriage support are cast aluminum and the entire instrument is finished in corrosion resistant white enamel.

The manometer is mounted on the tower by means of spring steel clips which give it full support and still permit easy removal for cleaning. The end of the manometer leading to the extraction chamber is held firmly in place by a bracket and plate assembly which prevents the transmission of vibration from the shaking assembly. The plate is held in place by thumb screws—eliminating the need for tools when removing the manometer from the superstructure.

The shaking assembly is ball-bearing mounted, to give smooth silent performance and is controlled by a wide range stepless variable transformer.

S-7325 BLOOD GAS APPARATUS — Van Slyke-Neill, All Metal, Sargent. Complete with Glassware. For operation from 115 volt 50/60 cycle circuits **$250.00**

SARGENT

SCIENTIFIC LABORATORY EQUIPMENT AND CHEMICALS

E. H. SARGENT & COMPANY, 155-165 EAST SUPERIOR ST., CHICAGO 11, ILL.
MICHIGAN DIVISION 1959 EAST JEFFERSON DETROIT 7, MICHIGAN
SOUTHWESTERN DIVISION 3915 PEELER ST. DALLAS 9, TEXAS

REAGENTS for the SEROLOGIC DIAGNOSIS of SYPHILIS

(All antigens are standardized by the author-serologists or by laboratories approved by them for this purpose.)

Complement-Fixation Reagents

Kolmer-Wassermann Antigen (Standard)
Kolmer-Wassermann Antigen (New)
Eagle-Wassermann Antigen
B-J-L Wassermann Antigen
5 cc. $3.50

Desiccated Complement
3 cc. $3.00
5 cc. 4.25
7 cc. 5.50
15 cc. 10.00

Anti-Sheep Hemolysin
(Desiccated)
1 cc. $2.00
(Glycerinated)
Equal parts of Glycerin and Hemolysin
2 cc. $2.00
10 cc. 7.50

Sheep Cells (Washed, Preserved 10% Suspension)
30 cc. $1.50
60 cc. 2.50
120 cc. 4.50

Sheep Blood (Sterile)
15 cc. $1.50
30 cc. 2.50
60 cc. 4.50

Positive Syphilitic Serum (4+)
(Desiccated)
2 cc. $3.00

Flocculation Reagents

Kahn Standard Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Kahn Sensitized Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Hinton Indicator
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Eagle Flocculation Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

B-J-L Flocculation Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Kline Antigen
1 cc. $2.50
5 cc. 10.00

1% Cholesterol in Alcohol
50 cc. $1.25
100 cc. 2.00

Mazzini Cholesterinized Antigen
4 cc. $2.50
(*With Sufficient Buffered Saline*)

CAPPEL LABORATORIES

BOX 352, WAYNE, PA.

• Kline Antigen is tested by an independent laboratory.

TESTING REAGENTS
for
Hematology and Serology

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Coombs' Serum
for antibody test

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and A_2

Human red cells
for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic serum
for control tests

P.S.A. (Paraffin Section Adhesive)

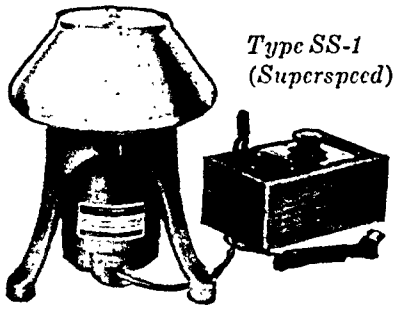
CERTIFIED BLOOD DONOR SERVICE

146-16 Hillside Avenue Jamaica 2, New York

SERVALL

ANGLE CENTRIFUGES

Ask for Bulletin J-31
with complete data on all models

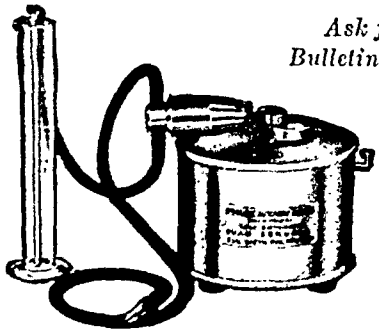


Type SS-1
(Superspeed)

With safety design self-centering device and dynamical balance. 13,000 r.p.m. 20,000 × G. Cap. 8 × 50cc or 15cc tubes.

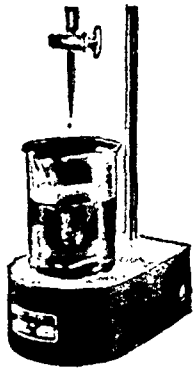
NEW TYPE SS-2 VACUUM CENTRIFUGE
15,000 r.p.m. 40,000 × G.
Capacity 16 × 50cc or 15cc tubes

Ask for Bulletin J-32



STERLING AUTOMATIC
AND
HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.
Easy to Operate—Time Saving



SERVALL
Magnetic Stirrer
(U. S. Patent)

Ask for Bulletin J-34

For stirring in open vessels or in closed systems under any pressure or under sterile conditions. Makes possible central rotation with vortex formation, epicyclic motion without vortex, simultaneous stirring in several small containers, etc.

Manufacturer and Distributor:
IVAN SORVALL, Inc.
210 FIFTH AVE.-NEW YORK 10, N.Y.

HELLIGE

COMPARATORS

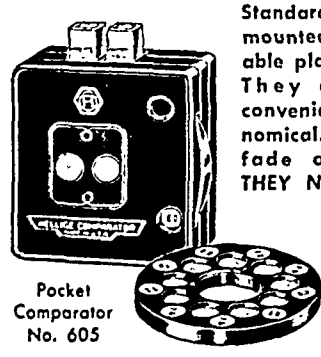


FOR BETTER

pH Control

there is nothing like

NON-FADING
GLASS COLOR
STANDARDS



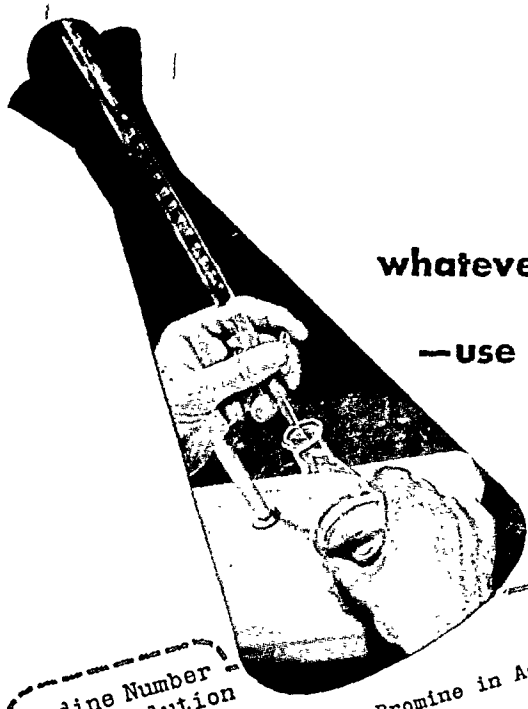
Pocket Comparator No. 605

Hellige Glass Color Standards are safely mounted in unbreakable plastic frames. They are more convenient and economical. They never fade or change, THEY NEVER FAIL.

WRITE FOR BULLETIN No. 602

HELLIGE
INCORPORATED
3718 NORTHERN BLVD., LONG ISLAND CITY 1, N.Y.
HEADQUARTERS FOR COLORIMETRIC APPARATUS

MALLINCKRODT



whatever you do

—use Mallinckrodt...

Use M.C.W. only!

Iodine Number
Hanus Solution

Solution of Iodine and Bromine in Acetic Acid.

Requires:
Iodine, A.R.
Glacial Acetic Acid, A.R.—dichromate tested.
Potassium Iodide, A.R.
Bromine, A.R.
Sodium Thiosulfate, A.R.

Standard Method in A.O.A.C. methods (1945) p. 494 (high precision). Also A.S.T.M. See also Snell and Biffen, p. 719; U.S.P. XIII, p. 839; Furman-Scott, p. 1800.

Prefer this to Wij's solution (uses Cl instead of Br).



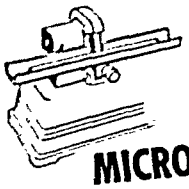
But whichever directions you choose, use Mallinckrodt Analytical Reagents. They are consistently the fine reagents A.C.S. specifications require. When your reagents bear the Mallinckrodt label you are sure of uniform dependable purity, and exceptional performance in every test.

ALWAYS SPECIFY MALLINCKRODT REAGENTS IN ORIGINAL PACKAGES

82 Years of Service to Chemical Users

MALLINCKRODT CHEMICAL WORKS

MALLINCKRODT STREET, ST. LOUIS 7, MO. • 72 GOLD STREET, NEW YORK 8, N.Y.
CHICAGO • CINCINNATI • CLEVELAND • LOS ANGELES • MONTREAL • PHILADELPHIA • SAN FRANCISCO
UNIFORM DEPENDABLE PURITY



the Berkeley
MICROTOME KNIFE SHARPENER
assures an
INCOMPARABLE EDGE
3 times faster . . .



COMPARE THE EDGES. Photomicrograph *(a)* shows a microtome knife edge sharpened by the Berkeley sharpener. Note its even smoothness, assurance of better sections. Photomicrograph *(b)* shows a blade laboriously hand sharpened, honed and stropped, but note the nicks and serrations still remaining.

COMPARE THE TIME. In tests, wherever made, and in practice in hundreds of laboratories, the Berkeley Microtome Knife Sharpener gives perfect edges in one-third the time required for hand honing and stropping.

Let us send you Bulletin MKS-12 giving full information about this indispensable laboratory aid.

Berkeley Scientific Company
SIXTH AND NEVIN AVE • RICHMOND, CALIFORNIA

SHILLABER'S
Non-Drying
IMMERSION OIL
for Microscopy

OFFERS THESE ADVANTAGES OVER CEDAR OIL . .

- *Better Results
- *Safer
- *Time Saving
- *Neutral
- *Low Fluorescence
- *2 Viscosity Ranges
- *Controlled Optical Properties



PRICES (Grade A or Grade B)

COMBINATION PACKET	
1 Ounce A with 1 Ounce B	$1.00
1 Ounce Bottle Each	.50
4 Ounce Stock Bottle	1.50
16 Ounce Stock Bottle	4.00

ONE OUNCE BOTTLES HAVE APPLICATOR RODS

Order Through Your Laboratory Supply Dealer Or Direct From

R. P. Cargille 118 Liberty Street New York 6, N. Y.

For maximum hormone adsorption in preparation of urine extracts for pregnancy testing use

"ADSORMONE"

A newly developed colloidal Adsorbing Agent

Rapid, simple technique
Eliminates drug reactions
No denaturing of hormone
More complete hormone adsorption
No animal loss from toxic urine
Costs less than 5¢ per test
For use with Frogs, Mice or Rabbits

For prices and information write

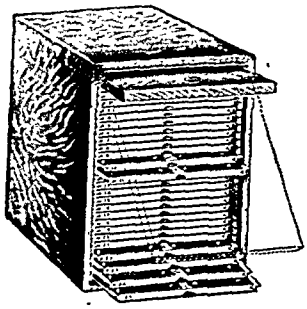
LOUIS C. HERRING & CO.
35 West Church Street
Orlando, Florida.



WHITE MICE
WHITE RATS



RESEARCH SUPPLY CO.
2436 W. York Street
Philadelphia 32, Pa.



Unit
System
Cabinet

No. 60-660
$36.00

EBERBACH
MICRO SLIDE CABINETS

The Unit System Cabinet provides *flat* storage for 500 3″ x 1″ slides. 25 aluminum trays are numbered, and each has 20 compartments. Hardwood cabinets with flat sides and disappearing door can be stacked as slide collection increases. They measure 8½ x 10 x 12″ deep. Write for Bulletin 150.

Eberbach & Son Company
Laboratory Apparatus & Supplies
Ann Arbor, Mich.

BBL
PHYTONE

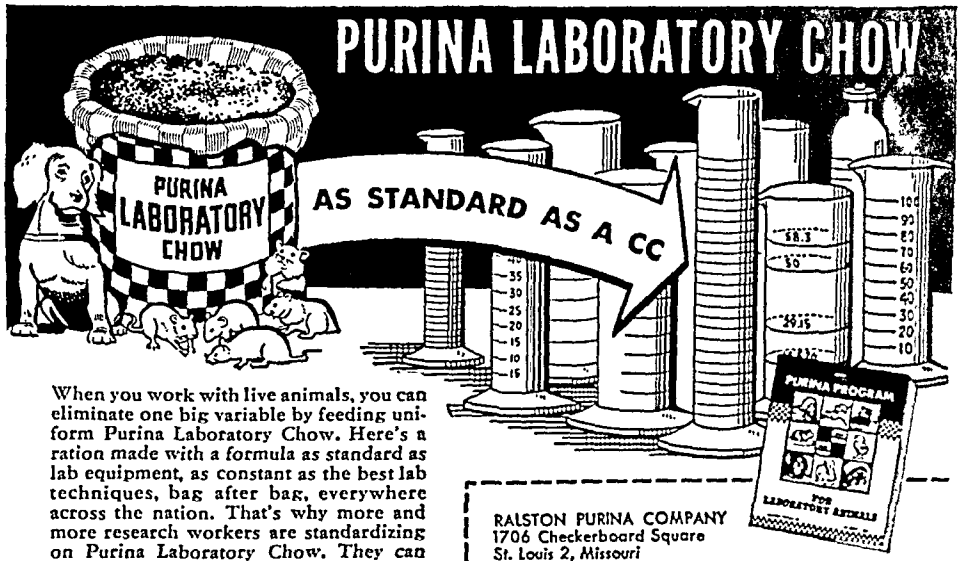
Papaic Digest of Soya Meal

An excellent peptone for the cultivation of fungi and bacteria, including such fastidious organisms as gonococci, pneumococci, clostridia and members of the genus Brucella.

A new B-B-L Catalog on PRODUCTS FOR THE BACTERIOLOGICAL LABORATORY is now available.
Copy will be sent upon request.

BALTIMORE
BIOLOGICAL LABORATORY
1640 Gorsuch Avenue Baltimore-18, Md.

PURINA LABORATORY CHOW



When you work with live animals, you can eliminate one big variable by feeding uniform Purina Laboratory Chow. Here's a ration made with a formula as standard as lab equipment, as constant as the best lab techniques, bag after bag, everywhere across the nation. That's why more and more research workers are standardizing on Purina Laboratory Chow. They can depend on it from year to year on their own projects, and they can rely on comparisons with other research scientists who use it on similar problems throughout the country. Try Purina Laboratory Chow . . . a standard you can count on.

RALSTON PURINA COMPANY
St. Louis 2, Mo.

RALSTON PURINA COMPANY
1706 Checkerboard Square
St. Louis 2, Missouri

Please send me your new 28-page handbook (SP4629) on the nutrition of laboratory animals.

Name_____

Address_____

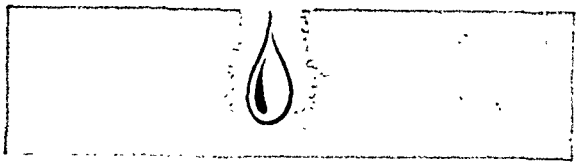
City_____ State_____

(Handbook available only in United States)

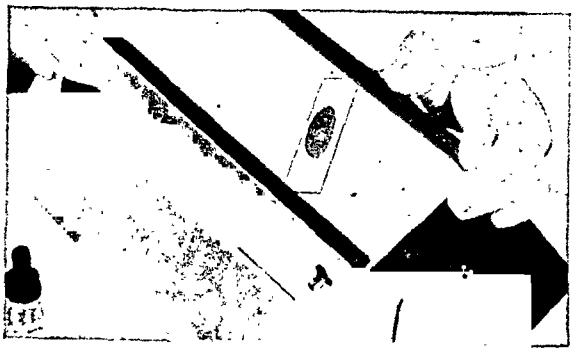
2 drops of blood...



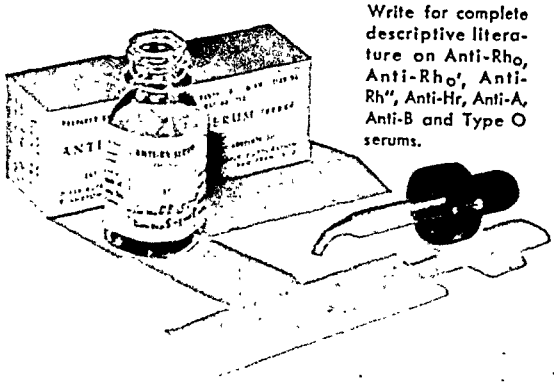
1 drop of Anti-Rh Serum...



Test complete in 2 minutes



Human Anti-Rh testing serums from American Hospital Supply Corporation are fast, potent, stable. They save time, eliminate errors. Slide method technique makes results clearly visible to the naked eye within 30 to 45 *seconds*. Prepared according to N. I. H. specifications.



Write for complete descriptive literature on Anti-Rho, Anti-Rho', Anti-Rh", Anti-Hr, Anti-A, Anti-B and Type O serums.

AMERICAN HOSPITAL SUPPLY CORPORATION

The *first name* in Hospital Supplies

GENERAL OFFICES—EVANSTON, ILLINOIS

Advertisers in the Journal Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.

NEW THIRD EDITION

A Textbook of

CLINICAL PATHOLOGY

Edited by the late

Francis P. Parker, M.D.

Formerly *Kracke and Parker*, the present edition has been almost entirely rewritten. New procedures developed in the last six years have been added, and rearrangement of sequence in some sections has improved the continuity.

Emphasis on interpretation of laboratory findings
Contributors all experienced workers
Wide range of topics in relatively small compass

Comprehensive Accurate Lucid

1043 pp., 229 figs., 46 plates, $9.00

The Williams & Wilkins Co.

Mt. Royal & Guilford Aves., Baltimore 2, Md.

(In writing to advertisers, please mention the Journal—it helps.)

"VACSEAL"

the Mark of Experience
in Guinea Pig Complement

Since 1939 most of the guinea pig complement in this country has been produced in our animal quarters and laboratory, but sold under another label.

Now the entire production of this material will be available under our own registered label, "Vacseal".

This will give the laboratory worker the advantage of a more favorable price. Standard uniformity is assured by our production method of pooling the serum from no less than 100 guinea pigs—an example of our long experience in producing satisfactory material.

"Vacseal" complement is packed in glass containers sealed under high vacuum. A special diluent is supplied which brings each package up to the following amounts:

3 cc 7 cc 20 cc

Write for trial 3 cc package, sent to any recognized laboratory on request. Prices on application.

Carworth Farms, Inc.

New City, Rockland County
New York

A Handbook for the Diagnosis of Cancer of the Uterus by the Use of Vaginal Smears

By Olive Gates, M.D.
and Shields Warren, M.D.

DUE in large part to the histological studies of the behavior of tumors under varying conditions, cancer has changed its position in the past two decades from the top of the list of hopeless, incurable diseases to a middle place among those diseases which are not only curable, but which, to a limited extent at least, may be prevented.

"This book should find a ready audience among physicians who, in increasing numbers, are becoming interested in the vaginal smear used as an adjunct of the existing means of diagnosing cancer of the uterine corpus and cervix. . . . It concludes with 50 excellent plates, each made up of from 2 to 6 photomicrographs showing the representative types of cells found in the vaginal smear."

—ARCHIVES OF PATHOLOGY

Second printing, 50 plates, tables, $4.00

At all bookstores, or from

Harvard University Press
Cambridge 38, Massachusetts

ATTENTION PATHOLOGISTS

As announced at the October 1947 meeting of the American Society of Clinical Pathologists, and in the January 1948 issue of the American Journal of Clinical Pathology, students beginning training in medical technology after January 1, 1948, must have such training in Approved Schools of Medical Technology, if they are working toward certification by the Board of Registry.

This announcement is repeated at this time, since some pathologists seem to be unaware of the new regulation.

The fifteenth edition of the Registry booklet of information is now available. The booklet contains complete information regarding all types of certification of laboratory workers, and other important rules and regulations. Copies will be sent free on request.

REGISTRY OF MEDICAL TECHNOLOGISTS

of the
American Society of Clinical Pathologists
Muncie, Indiana

(In writing to advertisers, please mention the Journal—it helps.)

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
STATE COUNSELORS 1949

Alabama.....ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona.....O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas.....M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California.....J. W. BUDD, 1407 S. Hope St., Los Angeles 15
P. MICHAEL, 434 30th St., Oakland 9
Colorado.....E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut.....R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware.....J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia...O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida.....L. Y. DRYENFORTH, 1022 Park St., Jacksonville 4
Georgia.....A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho.....J. A. BEEMAN, 309 Idaho St., Boise
Illinois.....O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana.....W. DODDS, Culver Hospital, Crawfordsville
Iowa.....F. H. LAMB, 220 Main St., Davenport
Kansas.....J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky.....H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana.....EMMA S. MOSS, Charity Hospital, New Orleans
Maine.....J. E. PORTER, 22 Arsenal St., Portland
Maryland.....C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts.....D. A. NICKERSON, Salem Hospital, Salem
Michigan.....C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota.....A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi.....R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri.....H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
V. B. BUHLER, General Hospital, Kansas City 8
Montana.....R. F. PETERSON, Murray Clinic, Butte
Nebraska.....J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada.....L. PARSONS, 235 W. 6th St., Reno
New Hampshire.....J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey.....S. A. GOLDBERG, Presbyterian Hospital, Newark
New York.....A. A. EGGSTON, 653 Park Ave., New York 21
V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina.....C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota.....L. W. LARSON, Clinic Bldg., Bismarck
Ohio.....F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma.....W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon.....V. D. SNEEDEN, U. of Oregon Mdeical School, Portland 1
Pennsylvania.....C. J. BUCHER, Jefferson Hospital, Philadelphia 7
T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island.....R. J. WILLIAMS, 64 Valentine Circle, Apponaug
South Carolina.....K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota.....R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee.....A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas.....J. J. ANDUJAR, 2951 Bentbrook Blvd., Fort Worth 4
Utah.....J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont.....E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia.....T. J. MORAN, The Memorial Hospital, Danville
Washington.....C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia.....F. C. HODGES, 800 First National Bank, Huntington
Wisconsin.....S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming.....S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Hawaii.....I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)

American Society of Clinical Pathologists

OFFICERS

President: Dr. O. A. Brines, Wayne University College of Medicine, Detroit 26, Michigan
Vice-President: Dr. C. H. Manlove, 5725 S.E. Stark Street, Portland 15, Oregon
President-Elect: Dr. J. B. McNaught, University of Colorado School of Medicine, Denver, Colorado
Secretary-Treasurer: Dr. C. G. Culbertson, 6060 Park Avenue, Indianapolis 5, Indiana

STANDING COMMITTEES

EXECUTIVE COMMITTEE

W. S. Thomas, Chairman, Rochester, N. Y.
T. J. Curphey, Long Island, N. Y.
F. W. Konzelmann, Atlantic City, N. J.
L. W. Larson, Bismarck, N. D
S. P. Reimann, Philadelphia, Pa.
J. R. Schenken, Omaha, Neb.

BOARD OF CENSORS

L. W. Diggs, Chairman, Memphis, Tenn.
J. J. Andujar, Fort Worth, Tex.
F. C. Coleman, Des Moines, Iowa
J. W. Howard, Wilmington, Del.
V. M. Johnson, West Palm Beach, Fla.
C. P. Larson, Tacoma, Wash.

BOARD OF REGISTRY OF MEDICAL TECHNOLOGISTS

L. G. Montgomery, Chairman, Muncie, Ind.
A. G. Foord, Pasadena, Cal.
F. B. Lynch, Jr., Philadelphia, Pa.
F. B. Queen, Portland, Ore.
R. G. Stillman, New York, N. Y.
A. H. Wells, Duluth, Minn.

APPOINTED COMMITTEES 1948–1949

BOARD OF CONSULTANTS FOR ENLARGING TUMOR REGISTRY

P. Klemperer
S. Otani*
S. P. Reimann
A. R. Rich*
F. W. Stewart*
A. P. Stout*
S. Warren
* Non-members

COMMITTEE ON EDUCATION AND PUBLICITY

W. O. Russell, Chairman
J. H. Black
M. Fein
W. M. Germain
D. A. Nickerson
S. P. Reimann

COMMITTEE ON NECROLOGY

H. R. Brown

COMMITTEE ON REGISTRY AND LOAN COLLECTIONS

F. J. Heck—Hematologic Division
J. W. Kernohan—Pathologic Division
E. S. Moss—Parasitologic Division

COMMITTEE TO STANDARDIZE EDUCATION AND TRAINING OF MEDICAL TECHNOLOGISTS

J. J. Andujar, Chairman
F. C. Coleman
O. O. Christianson
R. Miller
E. Moss
M. Oosting
P. Rosenblatt
J. P. Tollman

COMMITTEE ON SEMINAR

I. Davidsohn, Chairman
P. R. Cannon
Gen. R. O. Dart
R. A. Moore

REPRESENTATIVES ON NATIONAL RESEARCH COUNCIL SUBCOMMITTEE ON ONCOLOGY

I. H. Perry
S. Warren

GENERAL RESEARCH COMMITTEE

M. Strumia, Chairman
A. H. Sanford
W. M. Simpson

PROGRAM COMMITTEE

C. G. Culbertson, Chairman
R. A. Moore, Co-Chairman
C. J. Bucher
F. B. Queen
F. W. Sunderman

COMMITTEE ON HEMATOLOGIC TERMINOLOGY

E. E. Osgood, Chairman
L. Berman
F. J. Heck
R. R. Kracke
L. R. Limarzi

REPRESENTATIVE ON BOARD OF GOVERNORS OF COLLEGE OF AMERICAN PATHOLOGISTS

T. J. Curphey

DIFCO

Penicillin Inactivation

with

BACTO-PENASE

Bacto-Penase is a sterile thermolabile biological extract possessing the capacity to neutralize the antibiotic properties of all known types of *penicillin*. It is non-toxic to microörganisms and is therefore particularly satisfactory for use in the sterility testing of *penicillin* or products containing *penicillin*, and in determining the microbial counts on *penicillin*-containing products. BACTO-PENASE is recommended as a penicillinase for use in official procedures for sterility testing of *penicillin*. It is also recommended for use in media for culturing organisms from blood and other body fluids containing *penicillin* and for estimation of *penicillin* levels in such fluids.

PENICILLIN STERILITY TESTS

BACTO-PENASE in a concentration of 1 ml. per 15 ml. of Bacto-Fluid Thioglycollate Medium inactivates 50,000 units of *penicillin* in 40-48 hours incubation or 10,000 units of *penicillin* in 18 hours incubation.

BLOOD CULTURE

BACTO-PENASE is recommended for use in media for culturing blood or other body fluids containing *penicillin*. It is suggested that 1 ml. of BACTO-PENASE be added to 100 ml. of culture media such as Bacto-Brain Heart Infusion with P.A.B. or Bacto-Brain Heart Infusion with P.A.B. and Agar for this purpose. Media so prepared will inactivate the maximum amount of *penicillin* and sulfonamides present in 10 ml. or less of blood or other body fluids, and will also inactivate up to 1000 units of *streptomycin*.

℣ ℣ ℣

Stability. BACTO-PENASE and media containing this penicillinase are stable when stored at refrigerator temperatures (2°-6°C.).

Bacto-Penase Concentrate. BACTO-PENASE is available in concentrated form as BACTO-PENASE CONCENTRATE, 1 ml. of which neutralizes 500,000 units of *penicillin*. BACTO-PENASE CONCENTRATE is recommended in the sterility testing of *penicillin*.

BACTO-PENASE and BACTO-PENASE CONCENTRATE are supplied in standard packages of one-half dozen and one dozen screw cap tubes of 20 ml. each.

Descriptive literature is available upon request.

DIFCO LABORATORIES

DETROIT 1, MICHIGAN

AMERICAN JOURNAL OF CLINICAL PATHOLOGY

OFFICIAL PUBLICATION OF
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

S. E. GOULD, *Editor*

ADVISORY EDITORIAL BOARD

W. A. D. ANDERSON
J. J. ANDUJAR
J. E. ASH
L. BERMAN
G. R. BISKIND
O. A. BRINES
I. DAVIDSOHN
E. A. GALL
W. M. GERMAIN
A. S. GIORDANO
G. GOMORI
F. W. HARTMAN
J. W. KERNOHAN
J. A. KOLMER
H. J. LINN
T. B. MAGATH
A. R. MORITZ
S. P. REIMANN
S. B. ROSE
A. H. SANFORD
E. W. SCHULTZ
F. W. SUNDERMAN
W. S. THOMAS
E. WITEBSKY

PUBLISHED MONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, U. S. A.

COPYRIGHT, 1949, BY THE WILLIAMS & WILKINS COMPANY
Made in United States of America

American Journal of Clinical Pathology

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is devoted to the prompt publication of original investigations and observations in clinical pathology and pathologic anatomy. The JOURNAL is published monthly, one volume of twelve numbers appearing each year. The Journal includes a Technical Section, published bi-monthly beginning with the January issue of each volume, which is reprinted separately as the TECHNICAL BULLETIN. Manuscripts, books for review, and all inquiries concerning material to be published, should be forwarded to:

S. E. Gould, M.D., Editor
American Journal of Clinical Pathology
Wayne County General Hospital
Eloise, Michigan

Manuscripts. Manuscripts must be submitted in an original typewritten copy with *all* written matter, including references, double or triple spaced. Articles which are timely, and those which are presented concisely, will be given preference. Manuscripts will be accepted for publication on condition that they are contributed exclusively to the AMERICAN JOURNAL OF CLINICAL PATHOLOGY. References must be listed in alphabetic sequence, each reference being arranged in the following order: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviations of name of periodical (see *Index Medicus*), volume in Arabic numerals, inclusive pages, year. References should be limited to articles mentioned in the text. The nomenclature for bacteria should be that given in the sixth edition of *Bergey's Manual of Determinative Bacteriology*.

Illustrations and *tables.* Special care should be given to preparation of illustrations and tables, including legends and explanatory titles. Photographs preferably should be of rectangular shape, not more than 5″ x 8″, printed on glossy paper and should be unmounted. The magnification of photomicrographs should be stated. Illustrations must possess sufficient technical excellence to be acceptable. The Journal will assume the cost of a limited number of illustrations in black and white, and of a limited amount of tabular material.

Reprints. A table, listing cost of reprints, together with an order blank, is sent with proof. No reprints are furnished gratis to contributors. Orders for reprints should be sent to the publishers.

Subscriptions. The subscription price for the AMERICAN JOURNAL OF CLINICAL PATHOLOGY is $8.00 per volume in the United States and countries within the postal union; $8.50 in countries outside the postal union. Subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by Dr. C. G. Culbertson, Secretary, 6060 Park Avenue, Indianapolis 5, Ind. The subscription price for the TECHNICAL BULLETIN only is $2.00 per volume; 50 cents additional per volume for countries outside the postal union. Subscriptions from non-members should be sent to the publishers or their agents.

Advertising. Correspondence concerning advertising and other business matters should be addressed to the publishers.

Publishers: **The Williams and Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.**

Agents: For Australia: Angus and Robertson, Limited, 89–95 Castlereagh Street, Sydney; for the British Empire, except Australia and Canada: Bailliere, Tindall and Cox, 8 Henrietta St., Covent Gardens, W. C. 2, London, England; for Canada: Wm. Dawson and Sons Ltd., 70 King Street East, Toronto.

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879.
Subscription price, $8.00 per volume. Single numbers, $1.00.

American Journal of Clinical Pathology

VOLUME 19 FEBRUARY 1949 NUMBER 2

CONTENTS

The Dynamic Equilibrium. P. R. CANNON..... 99

An Outline for the Identification of Salmonellas. O. FELSENFELD..... 106

Aminopterin (A Folic Acid Antagonist) in the Treatment of Leukemia. L. M. MEYER, H. FINK, A. SAWITSKY, M. ROWEN AND N. D. RITZ..... 119

Use of a Folic Acid Antagonist in Chronic Leukemia. L. BERMAN, A. R. AXELROD, E. C. VONDER HEIDE AND E. A. SHARP..... 127

Further Evidence for Antibodies of Third Order. Fractionation of Agglutinins, Blocking Antibodies and Cryptagglutinoids by Physicochemical Methods. J. M. HILL, S. HABERMAN AND R. GUY..... 134

A Method for the Quantitative Measurement of Complement-Fixation. E. E. ECKER AND C. W. HIATT..... 141

Pooled Sheep Cells for Use in the Hemolytic System of the Complement-Fixation Test. T. F. MURASCHI AND V. N. TOMPKINS..... 152

Specificity of Cardiolipin Antigens in Tests for Syphilis. S. C. MCDEARMAN AND J. E. COTTRELL..... 156

Studies on Phagocytosis. II. R. ALTSCHUL AND A.M. FRIESEN..... 163

Phosphatases in Blood of Man. Values in Whole Blood, Plasma, Cytolysates and Erythrocytic Suspensions. H. BEHRENDT..... 167

Clinicopathologic Conference. G. A. BENNETT AND J. R PAUL..... 177

Selected Abstracts..... 183

Book Reviews..... 192

Obituaries..... 199

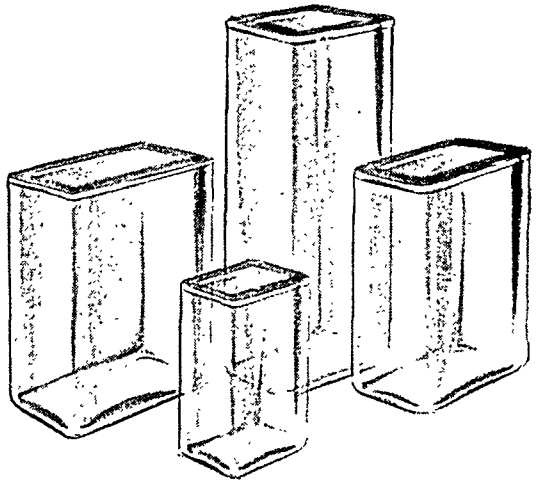
Supplement. The American Board of Pathology. List of Certified Pathologists..... 1

Know Your Product

RECTANGULAR MUSEUM JARS

Are they available again?

Yes, of course they are the *original* Bohemian Crystal Brand *Rectangular* Museum Jar.



WHY RECTANGULAR?

Only the rectangular shaped museum jar affords maximum visibility . . . elimination of curvature distortion . . . easy storage and handling and the durability required for permanent preservations.

WHY BOHEMIAN CRYSTAL BRAND GLASS?

This perfect crystal clear glass, free of refractive and distortive qualities is of prime importance to easy and exacting specimen inspection.

WHAT IS THE ADVANTAGE OF UNIFORM WALL THICKNESS?

Uniform wall thickness eliminates distortion and greatly increases the exactitude of the 4 sided perspective, allows a 100% visibility area which in turn permits multiple and simultaneous specimen inspection.

WHAT ARE THE FEATURES OF A PERFECT MUSEUM JAR?

1. Rectangular shaped.
2. Bohemian Crystal Brand lead glass. . free of striations and air bubbles.
3. Uniform wall thicknesses.
4. Flat bottoms for stability.
5. Exact fit covers with vent holes for easy, quick refilling with preserving fluids.
6. Availability in sizes to meet any requirement.

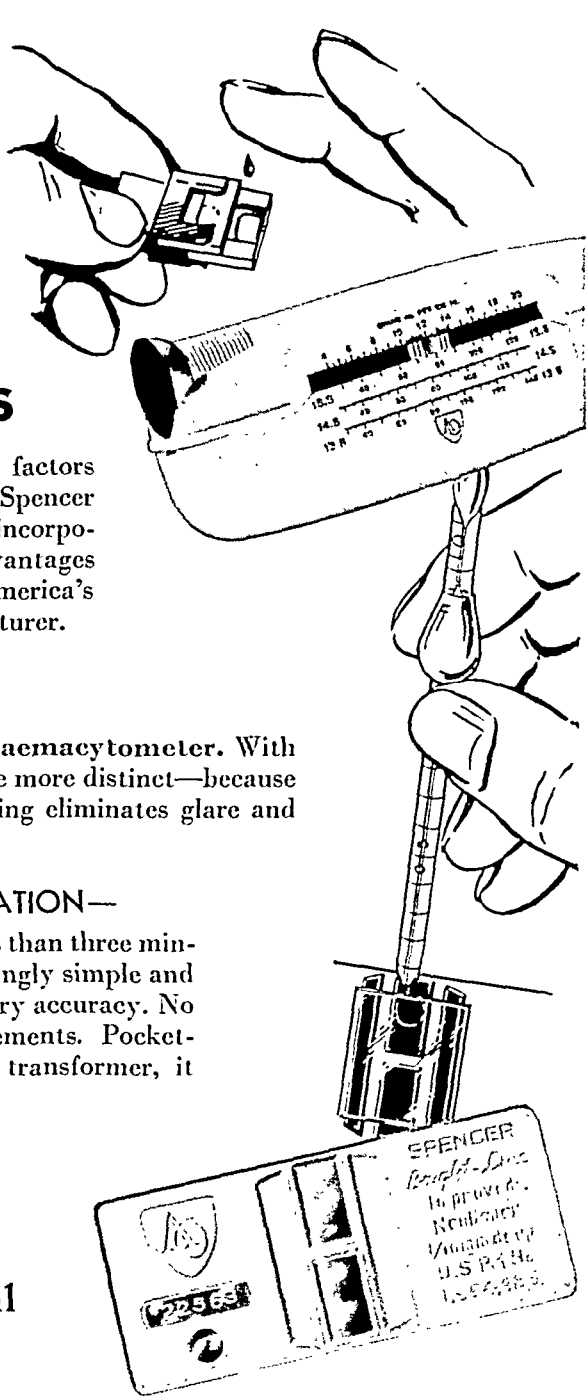
Propper

Mfg. Co., Inc., L. I. C., N[redacted]

The Propper name is your guarantee of greater value.

(In writing to advertisers, please mention the Journal—it helps.)

You're sure with SPENCER



BLOOD INSTRUMENTS

Accuracy and speed, the two vital factors in diagnosis, are easier to achieve with Spencer Blood Instruments—because they incorporate important and exclusive advantages developed by the research staff of America's *pioneer* optical instrument manufacturer.

FOR BLOOD COUNTING—

The Spencer "Bright-Line" Haemacytometer. With it, both blood cells and rulings are more distinct—because a semi-transparent metallic coating eliminates glare and increases visibility.

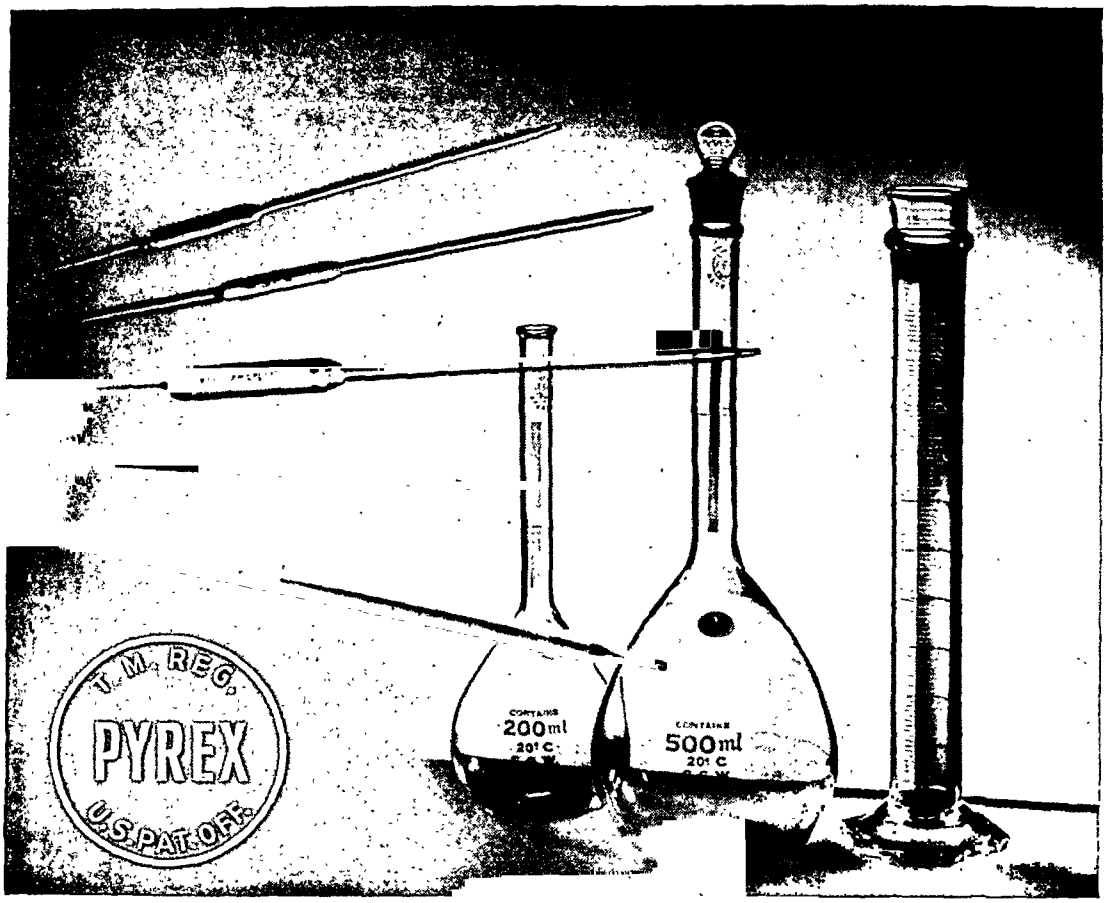
FOR HEMOGLOBIN DETERMINATION—

The Spencer Hb-Meter. In less than three minutes, using a method that's amazingly simple and error-free, you'll achieve laboratory accuracy. No dilutions or volumetric measurements. Pocket-sized, using either batteries or transformer, it may be operated anywhere.

See your local supplier or write Dept. 016.

American Optical
COMPANY
Scientific Instrument Division
Buffalo 15, New York

Manufacturers of the SPENCER *Scientific Instruments*



The BIGGEST VALUE in VOLUMETRIC WARE!

Corning does many things to give you the biggest value in volumetric ware. Made of PYREX brand glass No. 774, it's tops in quality. This pays off in longer service life and in greater accuracy.

In its chemical stability and resistance to thermal and physical shock, PYREX volumetric ware is outstanding. You can use it for so many different applications that it gives you greater working flexibility.

The design of this ware is also unique. PYREX brand cylinders are made with hex bases. They can't roll. Some types are made with reinforced tops. This minimizes breakage from accidental tipping. Each piece is individually calibrated for accuracy. This all adds up to bigger value.

And, PYREX volumetric ware is available with or without "Lifetime Red" graduations. When you specify "Lifetime Red," you know that the graduations will be permanent and *always* can be read. So when you order volumetric ware from your Corning Laboratory Supplier, specify PYREX brand and get extra value.

CORNING means research in GLASS

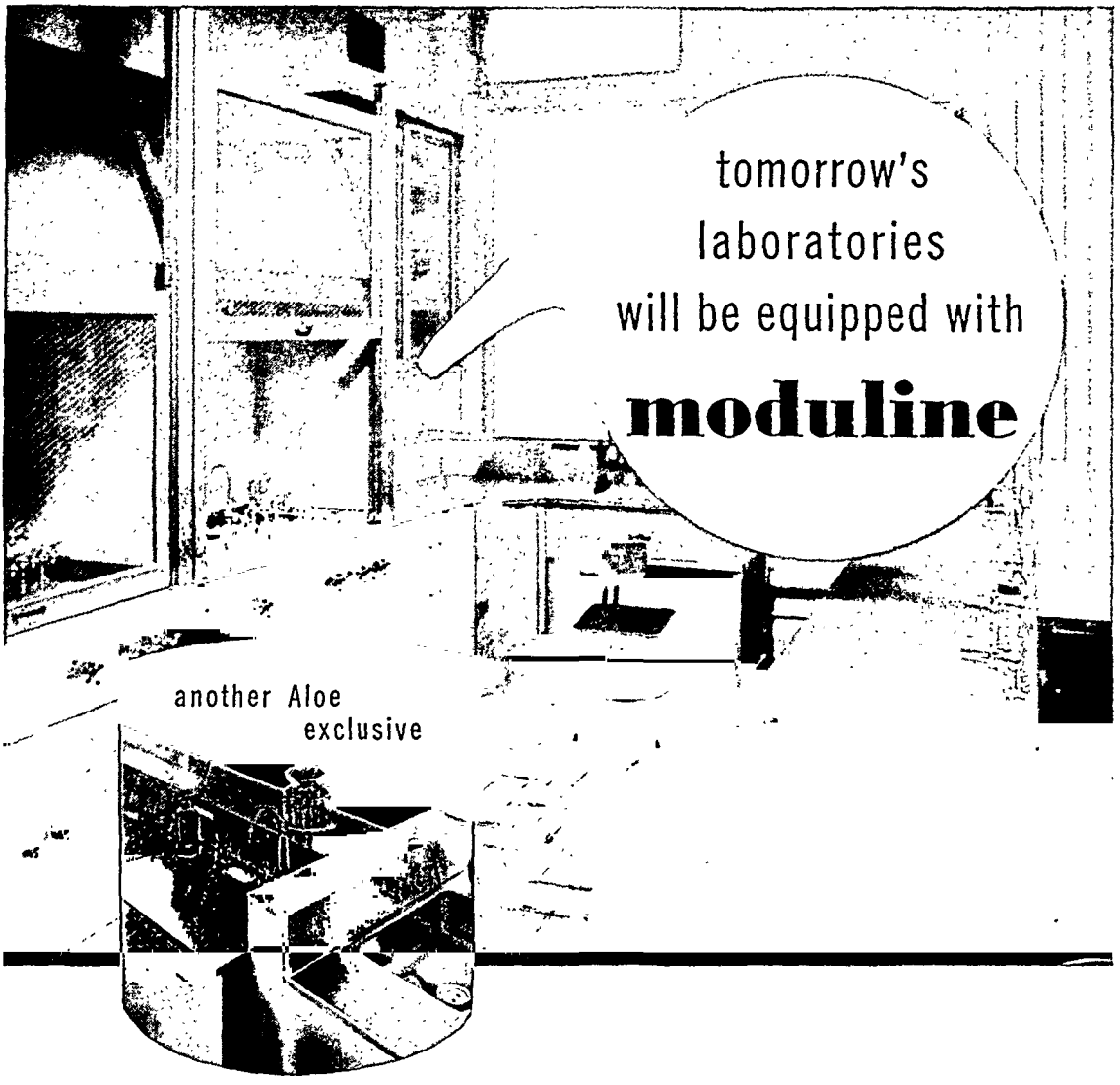
CORNING GLASS WORKS, CORNING, N. Y.

LABORATORY GLASSWARE

SEE OUR EXHIBIT
CHICAGO COLISEUM
NATIONAL CHEMICAL EXPOSITION
OCT. 12, 13, 14, 15, 16 1960

Stocked by Leading Laboratory Supply Houses

TECHNICAL PRODUCTS DIVISION: LABORATORY GLASSWARE • GLASS COMPONENTS
GLASS PIPE • GAUGE GLASSES • LIGHTINGWARE • SIGNALWARE • OPTICAL GLASS



the most modern laboratory furniture in the world

Moduline, by Aloe, comes in architectural approved widths and depths so that custom-built laboratory facilities may be developed from standard Moduline units. Notice these details: concealed hinges; baked steel finishes with stainless steel table tops; Furnished with or without reagent shelves. Utilities can be top or splashback mounted. No working space is taken up with utilities. Each new installation is convincing more persons that Moduline is the most functional, practical, laboratory furniture ever designed. Write for special booklet T-300 and learn how Moduline can help solve your furniture problems.

Special schematic layouts for laboratories available on request.

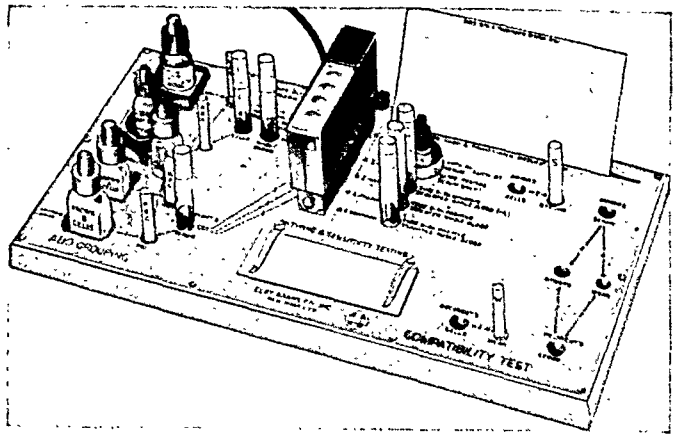
A. S. ALOE COMPANY *Serving Science Since 1860*

aloe

1831 Olive Street • St. Louis 3, Missouri

(In writing to advertisers, please mention the Journal—it helps.)

BROWN Blood Grouping and Cross Matching BOARD



- **Minimizes the possibility of technical or clerical errors.**
- **Embodies all the latest approved technics of blood grouping, Rh typing, Rh sensitivity testing and cross matching for transfusion.**
- **Simplifies and combines these technics in an orderly sequence.**
- **Facilitates the grouping and cross matching of blood for emergency or routine use.**
- **Can be used for routine Rh typing and sensitivity testing in individual maternity cases.**

The Brown Board[1] was developed in the Duke University Hospital Blood Bank to increase efficiency and reduce errors in blood typing and cross matching. It consists of an etched and stamped metal plate on a hardwood base, with an Rh typing box, anti-serum bottle adapters, reagent bottles, test tubes, dropping pipettes and concavity slides for Rh typing.

Tubes and reagent bottles have in-the-glass labels in different colors, matching the labelling and coloring of their places in the Board. Each reagent bottle and cross matching tube is of a different shape or size, so that it fits only in its proper place. Antiserum bottle adapters hold any commercial anti-serum bottle; they are permanently marked, fit only in their own place in the Board and are colored to conform to the coloring recommended for the sera by the National Institute of Health. Reagent bottles and adapters are designed to permit one-hand removal and replacement of the dropping pipettes.

The Rh typing box[2] is lighted, tiltable and designed to maintain a temperature of 37° to 42° C in the slide concavity where the blood sample is placed.

The Board has a slot to hold the Donor Card and Transfusion Request Form directly in front of the technician while tests are being made. A blotting paper holder is also provided, conveniently near the technician.

Refs: 1. "A Note on Blood Grouping and Cross Matching with Special Reference to a Convenient Cross Matching Board." Brown, I. W., Jr., M.D. In press.

2. "The Demonstration of Anti-Rh Agglutinins—An Accurate and Rapid Slide Test," Louis K. Diamond, M.D., Boston, Mass., and Neva M. Abelson, M.D., Philadelphia, Pa., J. of Laboratory and Clinical Medicine, vol. 30, No. 3, March 1945.

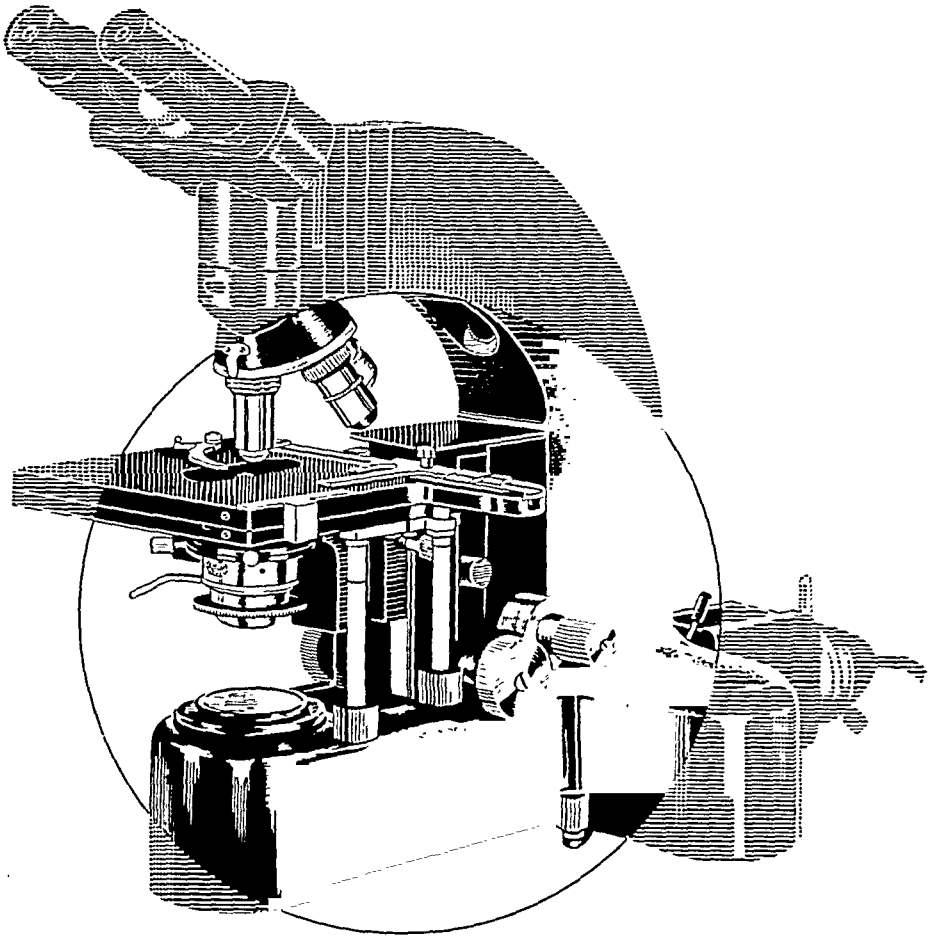
A-2800 Brown Blood Board, complete with Rh typing box, anti-serum bottle adapters, reagent bottles, test tubes, dropping pipettes and four-concavity slides. **Each $90.00**

CLAY-ADAMS COMPANY, INC.
141 EAST 25th STREET · NEW YORK 10
Showrooms also at 308 West Washington Street, CHICAGO 6, ILL.

(In writing to advertisers, please mention the Journal—it helps.)

Leitz

ORTHOLUX II RESEARCH MICROSCOPE



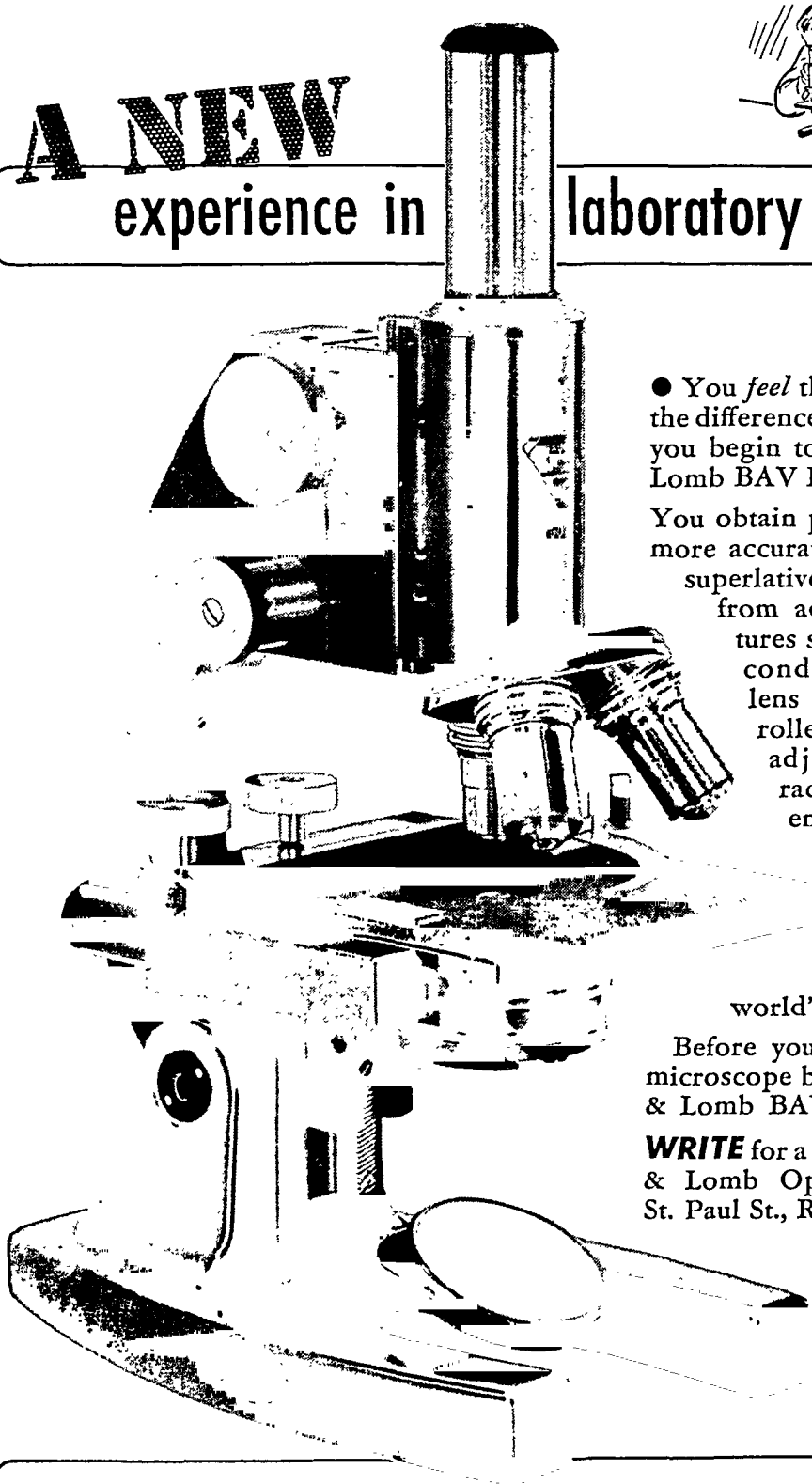
This outstanding Research Microscope, with built-in illumination, is made by Leitz—manufacturers of precision scientific instruments for 100 years. Write for Catalogue 600

E. LEITZ INC., 304 HUDSON STREET, NEW YORK 13, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)



A NEW experience in laboratory microscopy



● You *feel* the difference . . . You *see* the difference . . . from the first moment you begin to focus a new Bausch & Lomb BAV Laboratory Microscope.

You obtain proper adjustment faster, more accurately, with less effort . . . superlative performance resulting from advanced mechanical features such as the variable focus condenser, threadless cell lens mounts, nosepiece with roller bearing stop, coarse adjustment with *matched* rack and pinion, and patented lever fine adjustment.

Most important of all, you *see* better. Images of unmatched clarity and sharpness are provided by the world's finest optical system.

Before you buy a new laboratory microscope be sure to see the Bausch & Lomb BAV.

WRITE for a demonstration to Bausch & Lomb Optical Company, 652-B St. Paul St., Rochester 2, N. Y.

the Bausch & Lomb BAV Laboratory Microscope

Announcing

"VACSEAL" Complement

by Carworth Farms

We have produced this product since 1939 and are now offering it for the first time under our own label—"Vacseal"—with consequent reduction in price.

Our long experience in producing guinea pig complement is your assurance of a satisfactory product. Every known precaution is used to preserve complementary activity. Desiccation is obtained by the standard Lyophile-Chryochem process. The product is packed in all glass containers under high vacuum. A special diluent is supplied with ampule.

VACSEAL Complement is supplied for restoration to the following amounts:

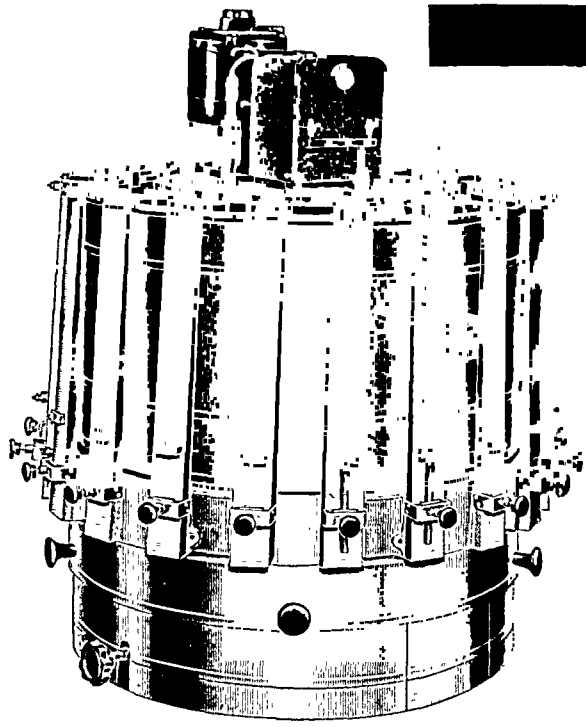
3 cc 7 cc 20 cc

A trial package will be sent to recognized laboratories on request.

CARWORTH FARMS, INC.
New City, Rockland County, New York

(In writing to advertisers, please mention the Journal—it helps.)

The "PRECISION" WARBURG APPARATUS



A TIME SAVING SPACE SAVING 20 UNIT STREAMLINED MODEL

★ Guaranteed Uniformity ±0.02°C. up to 60°C.
★ Noiseless Shaking Mechanism.
★ Manometer Can Be Read While Being Shaken
★ Adjustable Amplitude & Oscillation Speed.

The new "Precision" Warburg apparatus, used primarily for cell respiration work, occupies a space only 27 inches square. The manometer assembly may be revolved so that each manometer, in turn, can be brought into reading position in front of a stationary operator. Shaking amplitude can be varied between zero and 4 cm., and speed between 50 and 150 oscillations per minute.

It has a stainless steel bath and is equipped with low-drift immersion heaters; ¼ H.P. "Octo-Mix" stirrer; ¼ H.P. variable "V" belt drive; "Micro-Set" thermo regulator; "Merc-to-Merc" relay in control box; line switch; special thermometer 25° to 40°C. in 0.1°C. divisions; 20 stainless steel Warburg manometer supports and two 10-unit stands for supporting manometers.

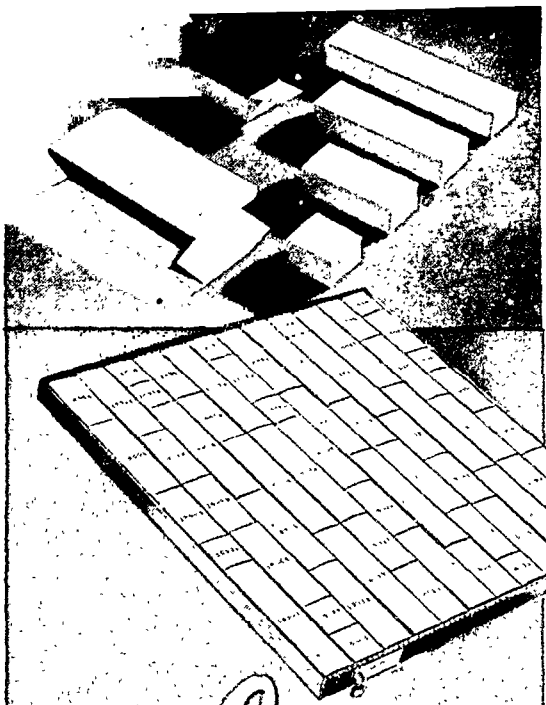
26460 - "PRECISION" WARBURG APPARATUS, 20 unit model, with standard equipment, 115 volts, 60 cyc. A.C., 2500 watts $750.00

DETAILED BULLETIN AND LITERATURE ON OTHER MODELS AVAILABLE ON REQUEST.

Write, Teletypewrite, Wire or Phone Dept. JCP-2, Our Nearest Office-Warehouse

SERVING SCIENCE THROUGH SUPPLY

WILL CORPORATION
LABORATORY APPARATUS AND CHEMICALS
ROCHESTER 3, N.Y. • NEW YORK 12, N.Y.
SOUTHERN SCIENTIFIC CO., INC., ATLANTA 3, GA.
BUFFALO APPARATUS CORP., BUFFALO 5, N. Y.



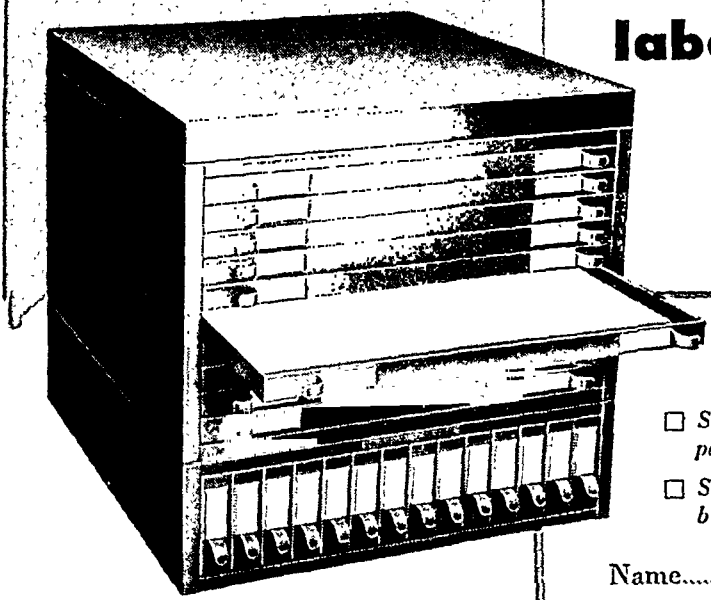
you needn't put up with "shoebox" block filing

Ask for a six-month-old paraffin block in many an otherwise well-ordered laboratory, and the hunt's on. Likely as not it means hours of prowling through a weird behind-the-scenes jumble of dusty, dog-eared cartons . . . trying everyone's temper, taking everyone's time, piling up hidden costs.

That's all out now, with this new "Lab-aid" block filing cabinet. You file your paraffin blocks in small, shipshape, white cardboard boxes, which come in varied sizes to accommodate all sorts of blocks, however irregular or out-sized. The boxes knock down, but spring to shape in a moment, so they're no storage problem. They're so proportioned that they fit snugly, row on row, in the shallow steel drawers.

The all-steel cabinet holds ten drawers, equipped with combination drawer-pulls and label-holders at each end. Each drawer will accommodate a gross of the 1½″ boxes: other sizes in ratio (see the filled drawers above). Multiply that by ten and you've some idea of the large capacity of this compact section (it's only 19″ square by 12″ high). That's because there's no waste space . . . *every cubic inch is a filing inch.*

the Lab-aid paraffin block file is another technicon laboratory essential



The "Lab-aid" paraffin block filing cabinet is finished to match all other types of "Lab-aid" files, and stacks interchangeably with any of them. Here it is stacked upon a vertical microslide file with 1″ drawers.

The "Lab-aid" laboratory filing system comprises a range of versatile equipment which brings new standards of filing efficiency to the pathological laboratory. For a descriptive bulletin, please use the coupon below.

The TECHNICON Company
215 E. 149th St., New York 51, N. Y.

☐ *Send me particulars of the new "Lab-aid" paraffin-block file.*

☐ *Send me complete "Lab-aid" filing system bulletin.*

Name.....

Address.....

City.....State.....

REAGENTS for the SEROLOGIC DIAGNOSIS of SYPHILIS

(All antigens are standardized by the author-serologists or by laboratories approved by them for this purpose.)

Complement-Fixation Reagents

Kolmer-Wassermann Antigen (Standard)
Kolmer-Wassermann Antigen (New)
Eagle-Wassermann Antigen
B-J-L Wassermann Antigen
5 cc. $3.50

Desiccated Complement
3 cc. $3.00
5 cc. 4.25
7 cc. 5.50
15 cc. 10.00

Anti-Sheep Hemolysin
(Desiccated)
1 cc. $2.00
(Glycerinated)
Equal parts of Glycerin and Hemolysin
2 cc. $2.00
10 cc. 7.50

Sheep Cells (Washed, Preserved 10% Suspension)
30 cc. $1.50
60 cc. 2.50
120 cc. 4.50

Sheep Blood (Sterile)
15 cc. $1.50
30 cc. 2.50
60 cc. 4.50

Positive Syphilitic Serum (4+)
(Desiccated)
2 cc. $3.00

Flocculation Reagents

Kahn Standard Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Kahn Sensitized Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Hinton Indicator
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Eagle Flocculation Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

B-J-L Flocculation Antigen
5 cc. $1.50
10 cc. 2.75
25 cc. 6.50

Kline Antigen
1 cc. $2.50
5 cc. 10.00

1% Cholesterol in Alcohol
50 cc. $1.25
100 cc. 2.00

Mazzini Cholesterinized Antigen
4 cc. $2.50
(With Sufficient Buffered Saline)

CAPPEL LABORATORIES

BOX 352, WAYNE, PA.

• Kline Antigen is tested by an independent laboratory.



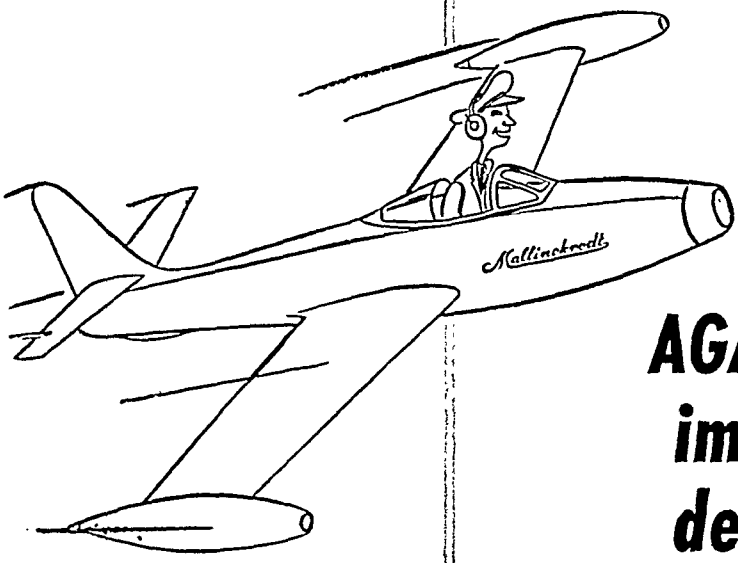
ACID ARSENIC, A.R.



ARSENIC TRIOXIDE, A.R.



ARSENIC PENTOXIDE, A.R.



AGAIN— immediate delivery

FINE CHEMICALS Mallinckrodt SINCE 1867

82 Years of Service to Chemical Users

Mallinckrodt Chemical Works

Mallinckrodt Street. St. Louis 7, Mo.
72 Gold Street, New York 8, N. Y.
Chicago • Cincinnati • Cleveland • Los Angeles
Montreal • Philadelphia • San Francisco

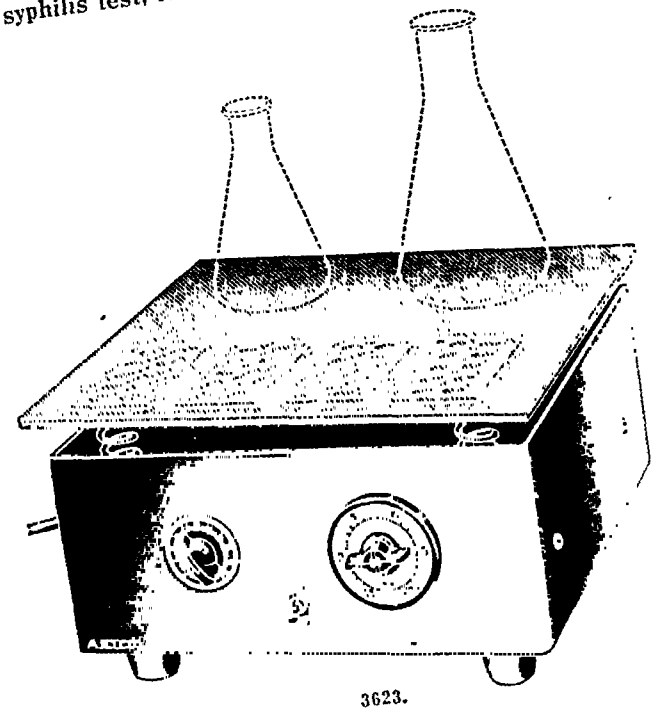
UNIFORM, DEPENDABLE PURITY

(In writing to advertisers, please mention the Journal—it helps.)

A.H.T. CO. SPECIFICATION

ELECTRIC ROTATOR

For use in the Kline, Boerner-Jones-Lukens, Mazzini and other flocculation tests, including the cardiolipin syphilis test, for shaking micro test slides, antigen flasks, etc.



3623.

ELECTRIC ROTATING APPARATUS, A.H.T. Co. Specification, with automatic cut-off timing device. For use in flocculation tests to eliminate the necessity for hand shaking of micro test slides, antigen flasks, etc. With rubber covered floating platform 13 inches square attached to four vertical springs fastened to the corners of a metal base, enclosed in a metal guard, and with an automatic cut-off timing device, speed control rheostat and 1/30 h.p. motor with worm reduction gear and driving mechanism which rotates the platform in a horizontal plane so that it circles an area ¾-inch diameter to simulate hand rotation as suggested in B. S. Kline, "*Microscopic Slide Precipitation Tests for Syphilis*", (*Baltimore, 1932*), *pp. 52–64.* See also Fred Boerner, Charles A. Jones and Marguerite Lukens, *American Journal of Clinical Pathology, Vol. 10, No. 11 (Nov., 1940), Tech. Sup., Vol. 4, No. 6, p. 141;* and L. Y. Mazzini, *American Journal of Clinical Pathology, Vol. 9, No. 2 (March 1939), p. 163.*

Range of speed, 70 to 210 r.p.m. Automatic timing device can be set for a maximum interval of 28 minutes in steps of ½ minute. Slides can be rotated safely simply by placing them on the surface of the rubber covered platform which is supplied with four retaining bands of rubber around the edges. Operation at a speed of approximately 200 r.p.m. for a period of four minutes best meets the requirements of both the Kline and Boerner-Jones-Lukens tests, and at 120 r.p.m. for four minutes meets the requirements of the Mazzini test. The motion duplicates almost exactly the excursion and vigor of hand shaking.

3623. **Electric Rotating Apparatus, A.H.T. Specification,** as above described, with automatic cut-off timing device, speed control rheostat with graduated dial with arbitrary scale from 0 to 100, four rubber retaining bands, cord and plug. For use on 115 volts, a.c. or d.c. 80.75

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

CABLE ADDRESS, "BALANCE," PHILADELPHIA

(In writing to advertisers, please mention the Journal—it helps.)

THE DYNAMIC EQUILIBRIUM*

PAUL R. CANNON, M.D.

From the Department of Pathology, The University of Chicago, Chicago, Illinois

I wish first to express my gratitude for the honor of having been chosen as this year's recipient of the Ward Burdick Award. I accept the award as an indication of the desire of this Society to commend the efforts of a group of which I am happy to have been the leader. It is especially gratifying to me, however, that the American Society of Clinical Pathologists, occupied as it is so largely with the many problems of practical pathology, should wish to honor those of us who, principally because of the good fortune of chance, have been able to direct a considerable share of our efforts toward some of the more theoretical aspects of pathology. I hope, moreover, that this dynamic organization may long continue to give its encouragement and support to unrestricted research in pathology, regardless of its immediately forseeable applications to medicine. I say this because I believe that probably in this way alone can we be best assured of the continued development and expanding influence of pathology, whether it is in relation to theory or to practice. For unless today's facts and ideas are constantly renewed and added to, they cannot serve tomorrow's needs in pathology any better in principle than can a camel's hump function indefinitely without dietary replenishment.

This afternoon I have chosen to discuss a subject, The Dynamic Equilibrium, for both a general and a special reason. We are living in a world of rapid changes and recurring tensions in which opposing forces come into equilibrium only momentarily. As a pessimistic theologian has recently said, "There is so little health in the whole of our modern civilization that one cannot find the island of order from which to proceed against disorder." To an optimist, however, these turbulences, the manifestations of a series of collisions between forces and counterforces must lead eventually to the kind of Progress which we all are trying to achieve. And to a *realist*, the main bulwark must be patience and hope. In our profession we are seeing similar conflicting trends. Since the early simple descriptive pathology of Morgagni, Rokitansky and Virchow, the scope of pathology has broadened and become more dynamic and complex until now almost every problem of medicine interests the pathologist, at least to some extent. From these complexities come the current problems and tensions of the pathologist as the patterns of practice change from day to day. One wonders whether these complexities are not the normal consequences of a rapid maturation of pathology, in the process of which we see the drives and conflicts, the ebb and flow, the give and take, of a dynamic equilibrium. In this process of rapid growth, this Society can take pride in the fact that a great deal of the

* The Ward Burdick Award Lecture, presented at the Twenty-Seventh Annual Meeting of American Society of Clinical Pathologists, in Chicago, October 13, 1948.

Received for publication, November 24, 1948.

acceleration, particularly in recent years, has resulted from the efforts of the clinical pathologist. Largely because of him the general pathologist no longer feels impelled to restrict his technic to the use of a single tool, the microscope, important as that instrument is and will continue to be to all of us. On the contrary, the general pathologist now utilizes more freely than ever before, and with increasing competency, the technics of chemistry, physics, mathematics, physiology, bacteriology, immunology, and other sciences, whenever and wherever they are indicated. Elsewhere,[4] in emphasizing this point I have stated that "it might be well to reflect that it was only because of the dynamic determination of the clinical pathologist that the shackles of custom have been so loosened within recent years that the latter is now free to utilize a wide variety of laboratory techniques in his daily work. Perhaps in time this technical revolution which the clinical pathologist has brought about may carry over into every field of pathology. With an increasing variety of techniques available it should be possible for pathologists to attack many additional problems of disease with ever greater resourcefulness."

But it is not my intention to carry further this general line of thought; instead, I wish to direct my principal discussion to a special aspect of the Dynamic Equilibrium as it applies to the processes of protein metabolism. For many years Dr. George H. Whipple and his associates have demonstrated in various ways the vital role in metabolism of the reserves of protein stored within cells throughout the body, reserves which contribute in time of need to the proteins of the circulating plasma.[10, 18, 19] Because of this relationship between circulating and tissue protein, there exists a nicely balanced or steady state, a dynamic equilibrium, between the protein stores and the plasma proteins, an equilibrium which can be modified, moreover, by variations in protein production or by protein wear and tear. These protein reserves may be reduced by fasting, low protein diet or plasma depletion; and this reduction becomes important to pathology because of its association with a lowered resistance to infection and intoxication, slower convalescence and impaired wound healing, and in other ways.

THE PROBLEM OF THE "PROTEIN POOL"

This concept of a dynamic equilibrium implies the presence in the tissues of a large "protein pool" which contributes to the plasma protein and the latter, in turn, to the functioning cells and tissues. In the concept of Whipple, plasma proteins act as a means of a fluid interchange between stores of "reserve" protein and cells in which protein is produced, modified and utilized; and, according to Terry, Hawkins, Church and Whipple,[14] "when albumins or globulins are formed rapidly within a *liver cell* because of body demand, the proteins presumably accumulate within the cell until there is an outflow—due to an increased density of intracellular protein or other factors. Conversely in protein fasting when parenteral plasma is given, these liver cells (and others) permit a protein inflow—perhaps due to a decreased density of intracellular protein." A further postulate in relation to this question of fluid interchange is that "pro-

tein molecules can *emerge* from a cell and also *enter* a cell readily—a dynamic equilibrium between cell and plasma protein."[19] But how this comes about is not clear, although Whipple suggests that intracellular enzymes may participate in the passage of plasma proteins through cell membranes.

Granting the validity of these assumptions, it follows that plasma protein functions directly as a circulating tissue and cellular nutrient without further degradation or transformation into its constituent amino acids. Plasma protein thus constitutes the nutritive core of tissue growth, repair and maintenance. It should be emphasized that nowhere has Whipple denied the possibility of the transformation or degradation of plasma protein into amino acids in the process of protein utilization; he has merely assumed that this is not metabolically necessary. The problem becomes of particular interest, however, because of the implication that the parenteral use of blood plasma can satisfy all the bodily needs for protein, even over considerable periods of time; and, as a matter of fact, there is good evidence that this is true, both from experiments in lower animals and in man. Thus Terry, Sandrock, Nye and Whipple[15] have been able to maintain two normal dogs in weight and nitrogen balance and in a state of well-being for at least three months by the daily intravenous or intraperitoneal injection of dog plasma, in association with an adequate diet very low in protein. It is of interest, however, that the concentration of plasma proteins gradually rose and that when it reached a concentration above 9.5 grams per 100 ml., proteinuria occurred. The authors suggest that this may indicate that the "utilization of parenteral plasma protein has a ceiling beyond which the body cannot use as efficiently this available plasma protein" after the body's protein reserves have been repleted. The fact, however, of the gradually developing hyperproteinemia is a direct evidence, also, of the slow absorption and utilization by the tissues of the injected protein, even in the earlier days of administration. This happens, moreover, in rats severely depleted of protein reserves by prolonged low-protein feeding (Fuller *et al.*[9]) and is probably an expression of the sluggish mechanism whereby the injected colloid leaves the blood and enters the tissues. This point will be considered in more detail in connection with the observations of Eckhardt *et al.*[7]

A DYNAMIC EQUILIBRIUM AT THE AMINO ACID LEVEL

But before accepting *in toto* the assumptions of this postulate of a *protein* equilibrium it is well to recall that at the amino acid level, too, there is evidence of the operation of a dynamic equilibrium. It is well known that during digestion the amino acid concentration of the blood may rise considerably, followed by the rapid removal and destruction of these amino acids by the liver. Some amino acids, however, pass through the liver; and in the fasting animal the amino acid concentration of the tissues may, for a time, be as much as ten times that of the blood plasma.[16] Soon, however, particularly in the liver, the amino acid concentration reverts to its original level as equilibrium with the blood is re-established. Since the work of Schoenheimer,[11] Borsook and Keighly,[3] and

du Vigneaud *et al.*,[17] it has become increasingly clear that the processes of protein metabolism are so dynamic that dietary amino acids almost immediately enter tissue proteins and freely interchange substituent groups. Thus, according to Schoenheimer,[11] the rapid and continuous chemical regeneration of the cell proteins characterizes living matter even though the composition of the tissues undergoes no final quantitative or qualitative changes. Tissue protein, therefore, can no longer be looked upon as a static structure. As Berg[1] has said, "The state which actually obtains is one of equilibrium, involving the summation of a complex array of interdependent equilibria between opposing dynamic forces." In view of this evidence of an active metabolism at low molecular levels it is necessary to examine further the question whether the dynamic equilibrium of the body's protein pool operates entirely or largely by a free interchange of large protein molecules. In other words, even though it is clear that plasma protein when administered parenterally can be utilized by the recipient for tissue protein synthesis, the *mechanism* of utilization still remains a problem of great interest and practical importance.

The following phenomenon is difficult to explain in terms of the free interchange of protein molecules: Although a normal well nourished rat requires a definite quantity per day of each of nine essential amino acids in order to stay in nitrogen equilibrium, if only slightly less than this amount of any one of these is omitted from the ration, the animal apparently cannot abstract, either from the circulating plasma or the tissues, even this small amount of essential amino acid and utilize it in association with other amino acids ingested in a ration adequate in each amino acid except the missing one.[5]

It is difficult to understand, with protein reserves available and protein aggregates present in abundance in the protein pool, why it should not be possible in times of metabolic need to supply from these sources such a small amount of needed amino acid. Evidently, however, there are no reserve stores of "free" essential amino acids; and in their absence, a deficiency in a particular one cannot be corrected from the protein stores themselves.

There is evidence, also, that the efficiency of utilization of hydrolyzed protein in the blood stream is correlated with its free amino acid content. It has been shown, for example, that completely hydrolyzed proteins are more efficiently utilized than are those in which there is a relatively high proportion of polypeptides. Thus Christensen, Lynch and Powers[6] found that when they compared the effects of intravenous injection of protein hydrolysate and of a solution of free amino acids, the former produced a larger and more prolonged elevation of plasma amino acid concentration and an associated higher urinary excretion of nitrogen. It is probable from these experiments, therefore, that the tissues are better able to utilize free amino acids than they are certain kinds, at least, of peptide aggregates. More recently Smyth, Levey and Lasichak[12] reported similar findings in human beings injected intravenously with protein hydrolysates. Here, too, the greatest urinary wastage of amino acid nitrogen followed the injection of a hydrolysate containing large amounts of peptide nitrogen. From these experiments it is difficult to see why, if the tissues cannot utilize

peptide nitrogen as efficiently as free amino acid nitrogen, they should be able to use larger molecular aggregates of plasma protein without the necessity of first breaking them down into constituent amino acids.

In a further study of the mechanism of protein utilization Eckhardt *et al.*[7] have offered an explanation which harmonizes both points of view. From observations made following the intravenous injection of human serum albumin in man, they have concluded that the albumin is not metabolized immediately but is slowly degraded into its constituent amino acids which later are utilized for purposes of tissue protein synthesis. These workers postulate that "whole protein (albumin) given intravenously to normal individuals diffuses first into the lymphatics where an equilibrium is established. It is then only slowly metabolized, finally breaking down gradually, and probably completely, into its constituent amino acids; these are either deaminated with excretion of the released nitrogen, or they are resynthesized into new tissue protein."

Because of the slow utilization of injected plasma protein, the latter enters the metabolic pool slowly, thus effecting a temporary increase in the concentration of total plasma protein and of the oncotic pressure of the blood. After the equilibrium between vascular and extravascular plasma protein has been established, however, slow degradation or decay allows release of amino acids for purposes of tissue synthesis. It should be noted that these observations were made in normal subjects; in severely malnourished subjects, it is not known at what tempo the enzymic degradation of plasma proteins might proceed.

THE SITE OR SITES OF TISSUE PROTEIN SYNTHESIS

Finally, what may be said about the relationship between circulating plasma proteins and the sites of synthesis of the proteins which constitute the protein pool? In the Whipple postulate, the liver is assumed to be the Master organ in protein synthesis. Among the reasons given for this assumption are the following: (1) The liver is strategically situated to perform this function; (2) it is of sufficient size to do so; (3) it has been proved that it "stores proteins, it makes proteins (fibrinogen, prothrombin and probably other globulins and albumins) and it aggregates amino acids and other nitrogenous materials coming from the gastrointestinal tract into proteins." To the stored protein within the liver cells, which can be released in time of need, Whipple[19] gives the name "transition protein" because he assumes that "by cleavage and reassembly (enzymes) it is on its way to cell protein or plasma protein." Only the protein which cannot be removed without cellular harm is called "indispensable protein". But, according to this concept, it is through the liver that the dynamic equilibrium with the circulating plasma proteins is maintained.

But is the liver the only place in which tissue proteins are fabricated or can protein synthesis occur also in other organs capable of converting an assortment of amino acids into polypeptides and complete tissue proteins? We have seen that during digestion large amounts of amino acids may pass through the liver and enter other tissues where, as for example in the muscles, they may be con-

verted into muscle protein. Although the evidence for this possibility is as yet meager, some support is at hand. For example, Bloch fed arginine labelled with N^{15} to rats and found the highest concentration of the isotopic nitrogen in the intestinal wall.[2] In explanation of this finding he suggested two possibilities: (1) Replacement of the isotopic nitrogen by ordinary nitrogen in the liver so that there is a low concentration of isotope in the liver as compared with that in the intestinal wall, or (2) a direct utilization of amino acids during the processes of absorption, protein synthesis occurring directly in the intestinal wall. More recently, Friedberg[8] has injected labelled methionine through the jugular vein and found the highest concentration of isotope in the intestinal mucosa. He found the same effect, also, when he injected tyrosine labelled with C^{14}. Tarver and Schmidt[13] had fed labelled methionine and found the highest concentration of S^{35} in the intestinal wall. Friedberg concludes from these experiments that there is a direct synthesis of proteins in the intestinal wall and as a consequence there is a replacement of enzymes and mucous proteins which are being lost. He suggests that for the same reasons there should be active synthesis in the pancreas, and re-emphasizes the findings of Tarver and Schmidt that this organ gave the second highest specific activity among the organs tested with isotopic amino acids.

If it is true, therefore, that amino acids can be directly utilized by synthesizing mechanisms in the intestinal mucosa, it would be logical to expect that the same should hold for other tissues as, for example, muscle and kidneys. No doubt by the use of isotopic methods more evidence will soon be available to help clarify this question.

In conclusion, enough is now known about these dynamic metabolic processes to afford a fairly clear picture of the over-all processes of protein anabolism and catabolism. Although the sharp distinction between "endogenous" and "exogenous" metabolism no longer holds, it is evident nevertheless that there is a daily expenditure of tissue protein, a daily tissue protein wear and tear, accompanied by a daily turnover rate for each essential amino acid. It is now evident, also, that both the tissue proteins and the dietary proteins intermingle freely and dynamically in the processes of intermediary metabolism; and the homeostatic mechanisms, operating presumably in an effort to maintain the constancy of the internal environment, tend to keep the metabolic processes in a state of dynamic equilibrium. As Claude Bernard emphasized many years ago with respect to the nature of the internal environment, it is its fixity which conditions a free and independent life, and all the living mechanisms, no matter how varied they may be, have as their sole object the maintenance of a constancy in the internal environment for all living conditions.

To some this may sound too purposive; but the fact remains that in the healthy organism these dynamic mechanisms seem to operate successfully and over long periods, in keeping the metabolic processes balanced and functioning smoothly and efficiently. Perhaps when our own understanding of the facts and theories now available to us becomes better balanced, our comprehension of these complicated processes may also come into equilibrium, even if this also be only for a moment.

REFERENCES

1. BERG, C. P.: Proteins and Amino Acids in Nutrition. Melville Sahyun, Editor. New York: Reinhold Publishing Corporation, 1948.
2. BLOCH, K.: Metabolism of 1 (+) arginine in rat. J. Biol. Chem., **165:** 469–476, 1946.
3. BORSOOK, H., AND KEIGHLY, G. L.: "Continuing" metabolism of nitrogen in animals. Proc. Roy. Soc., London, Series B, **118:** 488–521, 1935.
4. CANNON, P. R.: Tissue synthesis as a problem in pathology. Proc. Inst. Med. Chicago, **17:** 2–8, 1948.
5. CANNON, P. R.: The problem of tissue protein synthesis. Fed. Proc., Fed. Biol. Societies, **7:** 391–396, 1948.
6. CHRISTENSEN, H. N., LYNCH, E. L., AND POWERS, J. H.: Conjugates, non-protein, amino acids of plasma; peptidemia and hyperpeptiduria as result of intravenous administration of partially hydrolyzed casein (Amigen). J. Biol. Chem., **166:** 649–652, 1946.
7. ECKHARDT, R. D., LEWIS, J. H., MURPHY, T. L., BATCHELOR, W. H., AND DAVIDSON, C. S.: Chemical, clinical and immunological studies on the products of human plasma fractionation; comparative studies on the nutritive value of orally and intravenously administered human serum albumin in man. J. Clin. Investigation, **27:** 119–134, 1948.
8. FRIEDBERG, F.: Activity of protein synthesis by intestine. Science, **105:** 314–315, 1947.
9. FULLER, J. B., ET AL.: Unpublished experiments.
10. MADDEN, S. C., and WHIPPLE, G. H.: Plasma proteins; their source, production and utilization. Physiol. Rev., **20:** 194–217, 1940.
11. SCHOENHEIMER, R.: The Dynamic State of Body Constituents. Cambridge, Mass.: Harvard University Press, 78 pp., 1941.
12. SMYTH, C. J., LEVEY, S., AND LASICHAK, A. G.: The effects of the rate of administration of amino acid preparations on urinary wastage of amino acid nitrogen in man. J. Clin. Investigation, **27:** 412–417, 1948.
13. TARVER, H., AND SCHMIDT, C. L. A.: Radioactive sulphur studies. J. Biol. Chem., **146:** 69–84, 1942.
14. TERRY, R., HAWKINS, D. R., CHURCH, E. H., AND WHIPPLE, G. H.: Proteinuria related to hyperproteinemia in dogs following plasma given parenterally. A renal threshold for plasma proteins. J. Exper. Med., **87:** 561–573, 1948.
15. TERRY, R., SANDROCK, W. E., NYE, R. E., JR., AND WHIPPLE, G. H.: Parenteral plasma protein maintains nitrogen equilibrium over long periods. J. Exper. Med., **87:** 547–559, 1948.
16. VAN SLYKE, D. D.: Physiology of amino acids. Science, **95:** 259–263, 1942.
17. DU VIGNEAUD, V., CHANDLER, J. P., COHN, M., AND BROWN, C.: The transfer of the methyl group from methionine to choline to creatine. J. Biol. Chem., **134:** 787–788, 1940.
18. WHIPPLE, G. H.: Protein production and exchange in body, including hemoglobin, plasma protein, and cell protein. Am. J. M. Sc., **196:** 609–621, 1938.
19. WHIPPLE, G. H.: Hemoglobin, Plasma Protein and Cell Protein; Their Production and Interchange. American Lecture Series No. 12. Springfield, Ill.: Charles C Thomas, 1948.

AN OUTLINE FOR THE IDENTIFICATION OF SALMONELLAS*

OSCAR FELSENFELD, M.D.

From the Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Illinois

The identification of the types of Salmonella, also called "serodiagnosis" and "Salmonella typing", has been simplified recently by a revision of the Kauffmann-White-Edwards schema of Salmonella antigens.[10] Many antigens, such as "3", "4", "10", "11", "z_1" and "z_2" were dropped. The subgroups of *S. arizona* and *S. ballerup* have been excluded, the former being considered the nucleus of a new genus,[4] and the latter being classified with the paracolon organisms. Many monophasic salmonellas are not listed as special types. *S. cardiff*, *S. italiana* and *S. simsbury* which arise from *S. thompson*, *S. panama* and *S. senftenberg* by variation, were not admitted. *S. zagreb*, *S. oregon* and *S. pueris* were joined with *S. saint paul*, *S. muenchen* and *S. newport*, respectively. *S. mexico* was not included. The resulting classification is more practical and permits easier identification of the type of Salmonella.

The classic manual of Salmonella typing by Edwards and Bruner[2] has been somewhat outgrown by the vast extension of Salmonella work during the past few years. Edwards and Bruner[3] described a number of monophasic strains which greatly facilitate Salmonella typing. A new schema of typing is, therefore, needed.

The present outline was evolved, taking into consideration the following factors: Pooled polyvalent serums are suggested for the identification of the genus Salmonella; monovalent serums are used, whenever possible, for the identification of the species. If organisms for the production of monovalent serums are not available, salmonellas belonging to less frequent groups are used for the production of typing serums, in order to avoid cross reactions with other antigenic factors. The number of Salmonella strains necessary for the preparation of the serums is kept at a minimum.

PREPARATION OF SERUMS

Polyvalent serums. The preparation of reliable polyvalent serums was described by Galton and Quan,[7] Felsenfeld and Young,[6] Ewing and Bruner,[5] Crossley *et al.*[1] and Seligmann *et al.*[12] For general use, we recommend two serums preserved with 1:10,000 Merthiolate.† The pools are prepared according to the principle of Seligmann *et al.*[12] The first polyvalent serum is a mixture of "O" serums prepared against *S. bredeney* monophasic type, *S. tennessee*, *S. virginia*, *S. javiana* and *S. uganda*. Amounts equivalent to the homologous titer of each serum are used. A sample is given in Table 1. The second polyvalent serum is a pool of "O" serums prepared against *S. rubislaw*, *S. worthington*, *S. orientalis*, *S. kirkee*, *S. cerro*, *S. minnesota* monophasic strain, *S. XXVIII:y*, *S. urbana*, *S. adelaide*, *S. inverness* and *S. champaign*, which are representatives of the F group of salmonellas.

* Received for publication, September 24, 1948.

† A product of Eli Lilly Co., Indianapolis, Indiana.

"O" serums. These serums are prepared by inoculating rabbits with 0.25, 0.5, 1.0 and 2.0 ml. of the antigens at four day intervals. Six days later the rabbits are bled and the serums preserved with 1:10,000 Merthiolate. The "O" antigens employed for the above inocula-

TABLE 1

PREPARATION OF A POOLED "O" SERUM

"O" SERUM AGAINST	ANTIGENIC FORMULA	TITER AGAINST HOMOLOGOUS ORGANISM USING SLIDE AGGLUTINATION	ML. OF SERUM TO BE USED IN POOL
S. bredeney	I,IV,XXVII,XII	1:40	2.5
S. tennessee	VI,VII	1:20	5.0
S. virginia	VIII	1:50	2.0
S. javiana	I,IX,XII	1:20	5.0
S. uganda	III,X	1:25	4.0

TABLE 2

PREPARATION OF "O" AGGLUTINATING SERUMS

GROUP	ANTIGENIC FORMULA	ORGANISM USED FOR PREPARATION	SINGLE FACTOR DESIRED	ORGANISM USED FOR ABSORPTION
A	I,II,XII	*S. paratyphi A*	II	*S. javiana*
B_1	(I),IV,(V),XII	*S. IV,V,XII:e,h*	IV,V	*S. javiana*
B_2	I,IV,XXVII,XII	*S. bredeney* monophasic	XXVII	*S. IV,V,XII:e,h*
C_1	VI,VII	*S. tennessee*	VII	*S. newport* var. puerto rico
C_2	VI,VIII	*S. virginia*	VIII	none necessary
D	(I),IX,XII	*S. javiana*	IX	*S. paratyphi A*
E_1	III,X	*S. uganda*	X	*S. selandia*
E_2	III,XV	*S. selandia*	XV	*S. uganda*
E_3	I,III,XIX	*S. senftenberg*	XIX	*S. uganda*
F	XI	*S. rubislaw*		
G	(I),XIII,XXIII	*S. poona*		
H	(I),VI,XIV,XXV	*S. florida*	XIV,XXV	*S. tennessee*
I	XVI	*S. orientalis*		
Other groups	XVII	*S. kirkee*		
	XVIII	*S. cerro*		
	XXI	*S. minnesota* monophasic		
	XXVIII	*S. XXVIII:y*		
	XXX	*S. urbana*		
	XXXV	*S. adelaide*		
	XXXVIII	*S. inverness*		
	XXXIX	*S. champaign*		

tions are produced by growing the organisms on tryptose or trypticase agar slants for twenty-four hours, then washing off the growth with enough saline to obtain a suspension matching MacFarland's nephelometric scale at #3, and boiling the suspension for two hours to destroy "H" antigens. Table 2 shows the organisms used for the production of "O" serums.

Absorbed serums are prepared in the following way: The organism used for absorption is grown on tryptose or trypticase agar plates. Five plates are necessary for the absorption of each ml. of serum. After twenty-four hours of incubation, the growth is washed off with sufficient saline to give 0.5 ml. of bacillary suspension from each plate. The bacillary suspensions and the serum are mixed and incubated for two hours at 50 to 52 C. Then the organisms are removed by centrifugation and sufficient Merthiolate added to bring up its concentration to 1:10,000.

Unabsorbed serums keep better than absorbed serums. It is advisable, therefore, to keep on hand unabsorbed serums and to prepare the single factor serums from time to time, *e.g.*, every three to six months.

"Vi" serum. A strain of *E. coli Vi* is grown on tryptose or trypticase agar. The growth is washed off with absolute alcohol after twenty-four hours. The alcoholic suspension is dehydrated *in vacuo*. Just before use, the dried powder is restored to its original volume with sterile saline, and 0.25, 0.5, 1.0 and 1.0 ml. of the restored suspension are injected into a rabbit at four day intervals.

"H" serums. These are prepared by inoculating rabbits with 0.25, 0.5, 1.0, 2.0 and 3.0 ml. of the antigens at four day intervals. Six days later the rabbits are bled and the serums preserved with 1:10,000 Merthiolate. The "H" antigens are prepared by growing the organisms in tryptose or trypticase broth for eighteen to twenty hours, then adding one drop of formol (40 per cent Formaldehyde c.p.,) to each 5 ml. of the broth. The antigens are ready for use three hours after formolization, and keep unchanged for several months.

Table 3 lists the organisms used for the preparation of "H" serums. Absorbed serums are often used. They are obtained by a procedure identical to that recommended for the preparation of absorbed "O" serums. Double absorption is frequently used. If such an absorption is necessary, the serum is absorbed first with one, then with the second organism. The expiration time of absorbed and unabsorbed "H" serums is the same as that of "O" serums.

All serums have to be checked carefully against all flagellar ("H") and somatic ("O") antigenic factors present in the organism which was used for the preparation of the serum. "H" serums have to be tested even more carefully than "O" serums. There must be a sufficient range permitting the exclusion of "O" agglutination. Unabsorbed "H" serums with titers below 1:2,000 against the homologous "H" antigens must not be used. "H" serums showing agglutination to a higher titer than 1:250 with "O" antigens are rejected.

SALMONELLA TYPING

Serologic tests alone do not place an organism in the genus Salmonella. Biochemical reactions have to conform with those required by the definition of this genus. These are: acid and, frequently also, gas formation from dextrose and mannitol; no reaction in sucrose and lactose; no indole formation; and a negative Voges-Proskauer test. Further biochemical tests are of value only in the differentiation of some members of the groups C_1 and D. These reactions are listed in Table 4.

Organisms showing the biochemical reactions of salmonellas are tested first with the two polyvalent serums using the slide method. Instead of an excavated slide, the bottom of a petri dish may be used to perform these spot tests (Weil[13]). All organisms belonging in the Salmonella groups "A" to "E" will react with the first polyvalent serum, except *S. typhosa* and *S. paratyphi C* with strong "Vi" antigens. *S. typhosa* does not form gas and produces only a small amount of hydrogen sulfide. *S. paratyphi C* shows also typical biochemical reactions which, in addition to producing agglutination with "Vi" serums, help to diagnose these organisms.

TABLE 3

PREPARATION OF "H" AGGLUTINATING SERUMS

ANTIGENIC FORMULA	ORGANISM USED FOR PREPARATION	SUBGROUP OR FACTOR DESIRED	ORGANISM USED FOR ABSORPTION	FACTOR DESIRED	ORGANISM USED FOR SECOND ABSORPTION
a	*S. paratyphi A*				
b	*S. minnesota* monophasic				
c	*S. cholerae-suis* monophasic				
d	*S. virginia*				
e,h	*S. IV,V,XII:e,h*				
f,g	*S. adelaide*	f	*S. california*		
g,m,t	*S. california*	g	*S. oranienburg*		
		m	*S. senftenberg*		
		t	*S. montevideo*		
g,p	*S. dublin*	p	*S. california*		
g,q*	*S. moscow*	q	*S. california*		
g,p,u*	*S. rostock*	u	*S. dublin*		
g,s,t	*S. senftenberg*	s	*S. california*		
i	*S. typhi-murium* monophasic				
k	*S. thompson* monophasic				
l,v	*S. bredeney* monophasic	v	*S. worthington*		
l,w-z	*S. worthington*	l,w	*S. poona*	w	*S. bredeney* monophasic
		z	*S. dar es salaam*		
l,z_{13}-1,5*	*S. uganda*	l,z_{13}	*S. lexington*	z_{13}	*S. bredeney* monophasic
l,z_{28}-1,5*	*S. javiana*	l,z_{28}	*S. lexington*	z_{28}	*S. bredeney* monophasic
r-e,n	*S. rubislaw*	r	*S. abortus-equi*		
y	*S. XXVIII:y*				
z_4, z_{23}	*S. cerro*	z_{23}	*S. tallahassee*		
z_4, z_{24}*	*S. duesseldorf*	z_{24}	*S. tallahassee*		
z_4, z_{32}*	*S. tallahassee*	z_{32}	*S. duesseldorf*		
d-z_6	*S. niloese*	z_6	*S. virginia*		
z_{10}-1,5	*S. lexington*	z_{10}	*S. uganda*		
z_{29}	*S. tennessee*				
1,2	*S. newport* var. puerto rico	2	*S. cholerae-suis* monophasic		
1,5	*S. cholerae-suis*	5	*S. newport* var. puerto rico		
z-1,6	*S. poona*	1,6	*S. worthington*	6	*S. newport* var. puerto rico
e,h-1,7	*S. selandia*	1,7	*S. IV,V,XII:eh*	7	*S. newport* var. puerto rico
l,w-e,n*	*S. dar es salaam*	e,n	*S. worthington*		
e,n,x	*S. abortus-equi*	x	*S. dar es salaam*		
k-e,n,z_{15}	*S. orientalis*	e,n,z_{15}	*S. thompson* monophasic	z_{15}	*S. dar es salaam*

* Only large typing centers require these serums.

N.B.: S. cholerae-suis with "c" antigen only is called monophasic, specific, while the same organism with "1,5" antigens only is called monophasic, nonspecific.

Many salmonellas belonging to the groups "F" to "O" will be agglutinated by the first polyvalent serum and *vice versa*, because of the numerous common antigenic factors.

After a positive agglutination test is seen with the polyvalent serums, the "O" antigens are determined. The slide method is generally preferred also for this procedure. One drop of the serum is mixed with one drop of the antigen, the slide (or the petri dish) tilted for one minute and the results read with the naked eye. Serums in dilutions of one-half of the final titer are used for this test.

TABLE 4

ANTIGENIC AND BIOCHEMICAL PROPERTIES OF SOME MEMBERS OF THE "C_1" AND "D" GROUP OF THE GENUS SALMONELLA

SALMONELLA	ANTIGENIC STRUCTURE	FERMENTATION OF			FORMATION OF H_2S
		Inositol	Trehalose		
S. paratyphi C	VI, VII:c-1,5	0	+		+
S. cholerae-suis	VI, VII:c-1,5	0	0		0
S. cholerae-suis monophasic, specific	VI, VII:c-	0	0		0
S. cholerae-suis monophasic, nonspecific	VI, VII:-1,5	0	0		+
S. typhi-suis	VI, VII:c-1,5	0	+		0
S. typhi-suis monophasic	VI, VII:-1,5	0	+		0
S. thompson	VI, VII:k-1,5	+	+		+
S. thompson monophasic, specific	VI, VII:k-	+	+		+
S. thompson monophasic, nonspecific	VI, VII:-1,5	+	+		+
		Dextrose	Dulcitol	Maltose	
S. pullorum	IX, XII	Acid + gas	0	Usually +	+
S. gallinarum	IX, XII	Acid	+	+	+ or 0

Organisms forming hydrogen sulfide and giving a positive agglutination test with the first polyvalent serum are tested with absorbed B_1, C_1, C_2, D and unabsorbed E_1 serums. If the reactions are negative or if hydrogen sulfide is not formed, A serum is used. If the reaction is positive with B_1 serum, a test is made with B_2 absorbed serum. If reaction occurred with unabsorbed E_1 serum, absorbed E_1, E_2 and E_3 serums are used. Since about 90 per cent of all salmonellas isolated in the United States are organisms belonging to groups B, C and D, much serum is saved if bacteria suspected of being salmonellas which form hydrogen sulfide are first tested with absorbed B_1, C_1, C_2 and D serums.

If the Salmonella does not belong in groups A to E, tests must be carried out with additional serums listed in Table 2. It must be kept in mind that many paracolon organisms will reveal partial or total salmonella "O" antigens. Careful biochemical tests will eliminate them.

There is a difference of opinion regarding the best way of preparing the salmonellas for "O" agglutination tests. Many workers use the growth from the surface of an agar slant, triple sugar iron medium, Kliegler's or Krumwiede's tube for spot-agglutination without further preparation. Others wash the surface of such a slant with some saline to obtain a heavy suspension, which is then boiled for one or two hours. When the organisms are slightly rough, it is necessary to wash the surface of the agar slants with some saline, centrifuge off the organisms, replace the saline with absolute ethyl alcohol, keep the suspensions in a water bath at 56 C. for two hours, centrifuge, drain off the alcohol, and then add sufficient saline to make up the original volume.

After the "O" antigens are determined, the "H" antigens have to be established. Tube agglutination is used for this purpose. The "H" antigen of the examined salmonella is prepared as the antigens for the production of "H" serums. The organisms are tested with only one dilution to each serum. It is customary to use serums in dilutions 1:1,000. Usually 0.5 ml. of the serum diluted 1:500 and 0.5 ml. of the "H" antigen are mixed and incubated at 50 to 52 C. in the water bath. The results are read after ten, thirty and sixty minutes.

Occasionally, one phase of biphasic salmonellas will be weak and it becomes necessary to enhance that phase by suppressing the other phase. For instance, an organism belonging to group B_1 may be agglutinated only by "1, 2" serum. Organisms with these antigenic factors are seldom monophasic, so that the other phase has to be elicited. This is achieved by growing the strain in the presence of "1,2" antiserum. This serum will suppress phase "1,2" and "bring out" the other phase. Several technics have been devised for this purpose. Edwards and Bruner[2] recommend the addition of one loopful of the suppressing serum to semisolid agar, thereby permitting the spread of the organism containing the other phase. Also Hajna,[8] Juenker[9] and Kuhn[11] have published excellent methods to achieve this end. We generally use the technic of Hajna.[8] A U-shaped tube is filled with semisolid agar (containing 0.5 per cent agar, 1.0 per cent tryptose or trypticase and 0.5 per cent sodium chloride, pH 7.2) with the addition of a small loopful of the suppressing serum. The medium is inoculated with the culture by barely penetrating the surface of the agar in one branch of the tube. After twenty-four hours' incubation the unsuppressed phase of the Salmonella may be isolated from the uninoculated arm of the U-tube and transferred to broth.

Table 5 indicates a procedure recommended for the typing of salmonellas. The determination of "O" antigens was discussed in the foregoing paragraphs. The "H" serums are grouped in series in such a way that more salmonellas will be detected by the use of serums listed in the first or second series. Much time, material and effort will be saved by beginning with the first series and setting up tests with serums of the second and consecutive series only when the reactions in the first group are negative. Salmonellas with suppressed phases may cause difficulties and lead to the temptation to use many additional serums. It may be advisable to use the third series of serums only after an attempt to elicit the suppressed phase has been made, especially when common salmonellas, such as *S. paratyphi B* or *S. typhi-murium*, are suspected.

TABLE 5

SALMONELLA TYPING

GROUP "O" SERUM	SERIES OF "H" SERUMS	SERUMS CONSTITUTING THE SERIES	IF POSITIVE, USE "H" SERUM
IV,V + XXVII −	(1)	b	2 = paratyphi B
			e,n,x = abony
		i	2 = typhi-murium
		f,g	f g m t
			+ + − − = derby
			− + + − = essen
			− + + + = california
			− + − + = budapest
		2	(b = paratyphi B)
			(i = typhi-murium)
			d = stanley
			e,h = st. paul
			l,v = oahu
			r = heidelberg
			y = coeln
	(2)	e,h	2 = st. paul
			5 = reading
			7 = kaapstad
			e,n,x = chester
			e,n,z_{15} = san diego
			d and e,n,z_{15} = salinatis
		l,v	(2 = oahu)
			e,n,z_{15} = brandenburg
		c	6 = abortus ovis
			7 = altendorf
		x	a = bispebjerg
			(b = abony)
			(e,h = chester)
			no reaction, H_2S negative = abortus equi
		5	e,h = reading
	(3)	a	7 = arechavaleta
			(e,n,x = bispebjerg)
		d	(2 = stanley)
			(z_{15} = salinatis)
		k	z_{15} = texas
		r	(2 = heidelberg)
		y	(2 = koeln)
		6	(c = abortus ovis)
		7	(a = arechavaleta)
			(c = altendorf)
			(e,h = kaapstad)
		z_{15}	(e,h = san diego)
			(l,v = brandenburg)
			(k = texas)
			(d and e,h = salinatis)

TABLE 5—*Continued*

GROUP "O" SERUM	SERIES OF "H" SERUMS	SERUMS CONSTITUTING THE SERIES	IF POSITIVE, USE "H" SERUM
IV, V + XXVII +	(1)	b	x = abortus bovis *negative, gelatine* + = schleissheim
		d	7 = schwarzengrund
		l,v	7 = bredeney
		7	(d = schwarzengrund) (l,v = bredeney)
		x	(b = abortus bovis)
VII +	(1)	c	5, check from Table 4
		g,m,s	s+ t− = montevideo
		m,t	s− t+ = oranienburg
		k	5, check from Table 4
			6 = daytona
			x = singapore
		5	c = paratyphi C or cholerae-suis, check from Table 4 k = thompson or typhi-suis, check from Table 4 r = infantis y = bareilly
	(2)	r	2 = virchow (5 = infantis) z_{15} = papuana
		y	2 = richmond (5 = bareilly) x = hartford z_{15} = mikawasima
		z_{29}	= tennessee
		2	l,v = concord (r = virchow) (y = richmond)
	(3)	a	x = oslo
		b	z_{15} = georgia
		d	(5 = mission) x = amersfoort
		e,h	6 = norwitch z_{15} = braenderup
		l,v	(2 = concord) z_{15} = potsdam
		6	(e,h = norwitch)
		x	(a = oslo) (d = amersfoort) (k = singapore) (y = hartford)
		z_{15}	(b = georgia) (e,h = braenderup) (l,v = potsdam) (r = papuana) (y = mikawasima)

TABLE 5—*Continued*

GROUP "O" SERUM	SERIES OF "H" SERUMS	SERUMS CONSTITUTING THE SERIES	IF POSITIVE, USE "H" SERUM
VIII +	(1)	d	2 = muenchen 5 = manhattan negative = virginia
		i	x = bonariensis z_6 = kentucky
		e,h	2 = newport 5 = kottbus
		r	5 = morbificans bovis z_{15} = hidalgo
		2	(d = muenchen) (e,h = newport) l,v = lichtfield
		5	(d = manhattan) (e,h = kottbus) (r = morbificans bovis)
	(2)	l,v	(2 = lichtfield) 6 = amherstiana
		z_4	z_{24} = duesseldorf z_{32} = tallahassee
		z_6	i = kentucky
		z_{10}	z_{15} = glosstrup
	(3)	a	x = narashino
		b	x = gatuni
		6	(l,v = amherstiana)
		x	(a = narashino) (b = gatuni) (i = bonariensis)
		z_{15}	(r = hidalgo) (z_{10} = glosstrup)
IX,XII +			No gas, some H_2S; d+ Vi+ or − = *S. typhosa* Non-motile, check from Table 4
	(1)	a	5 = sendai e,n = loma linda z_{15} = durban
		l,v	5 = panama w and e, n = dar es salaam z_{13} = napoli (confirm x+) z_{15} = goettingen z_{28} = javiana
		g,m,t	f m p q t u + − − − + − = berta − + − − − − = enteritidis − + − + − − = blegdam − + − − + − = pensacola − − + − − − = dublin − − + − − + = rostock − − − + − − = moscow

TABLE 5—*Continued*

GROUP "O" SERUM	SERIES OF "H" SERUMS	SERUMS CONSTITUTING THE SERIES	IF POSITIVE, USE "H" SERUM
IX,XII + (*Cont.*)		5	(a = sendai) e,h = eastbourne k = claibornei (l,v = panama) (l,z_{18} = javiana) z_{29} = canastel
	(2)	b e,h k r z_{29}	2 = onarimon (5 = eastbourne) (5 = claibornei) x = shoreditch (5 = canastel)
	(3)	2 e,n x z_{15}	(b = onarimon) (a = loma linda) (l,w = dar es salaam) (l,z_{13} = napoli) (r = shoreditch) (a = durban) (l,v = goettingen)
X,XXVI +	(1)	e,h l,v 5 6 7	2 = vejle 5 = muenster 6 = anatum 7 = nyborg l,w = meleagridis 5 = uganda (confirm z_{13}+) 6 = london 7 = give (e,h = meleagridis (confirm w+)) b = mission d = shangani (e,h = muenster) k = zanzibar (l, z_{13} = uganda) y = orion z_{10} = lexington (e,h = anatum) (l,v = london) z_6 = saipan (e,h = nyborg) (l,v = give)
	(2)	r w 2 z_6 z_{10}	z_6 = weltevreden (e,h = meleagridis) (e,h = vejle) y = amager (r = weltevreden) (6 = saipan) (5 = lexington)

TABLE 5—*Continued*

GROUP "O" SERUM	SERIES OF "H" SERUMS	SERUMS CONSTITUTING THE SERIES	IF POSITIVE, USE "H" SERUM
X,XXVI (*Cont.*)			
	(3)	b	(5 = mission)
		d	(5 = shangani)
		k	(5 = zanzibar)
		y	(2 = amager)
			(5 = orion)
XV +	(1)	e,h	w = cambridge (confirm: v−)
			6 = newington
			7 = selandia
		v	7 = new brunswick (confirm: w−)
		w	(e,h = cambridge)
		5	z_{10} = illinois
		6	(e,h = newington)
		7	(e,h = selandia)
			(v = new brunswick)
		z_{10}	(5 = illinois)
XIX +	(1)	d	z_6 = niloese
		g,s,t	senftenberg
		i	z_6 = taksony
		z_6	(d = niloese)
			(i = taksony)
II +		a	paratyphi A
XI +	(1)	i	2 = aberdeen
			e,n = veneziana
		k	2 = pretoria
		r	5 = senegal
			e,n = rubislaw
		2	(i = aberdeen)
			(k = pretoria)
		e,n	(i = veneziana)
			(r = rubislaw)
	(2)	a	5 = marseille
			z_{15} = luciana
		y	5 = solt
		5	(a = marseille)
			(r = senegal)
			(y = solt)
		z_{15}	(a = luciana)
XIII +	(1)	f,g	havana
		w	z = worthington
		z	(w = worthington)
			6 = poona
		6	(z = poona)
			v = borbeck
		z_{29}	cubana

TABLE 5—*Concluded*

GROUP "O" SERUM	SERIES OF "H" SERUMS	SERUMS CONSTITUTING THE SERIES	IF POSITIVE, USE "H" SERUM
XIII + (*Cont.*)			
	(2)	b	5 = mississippi
		d	7 = grumpensis
			none = wichita
		v	(6 = borbeck)
		5	(b = mississippi)
		7	(d = grumpensis)
XIV,XXIV +	(1)	e,h	5 = onderstepoort
		y	7 = carrau
		5	d = heves
			(e,h = onderstepoort)
		7	d = florida
			(y = carrau)
	(2)	d	(5 = heves)
			(7 = florida)
		l,v	x = horsham
		z	x = sundsvall
		x	(l,v = horsham)
			(z = sundsvall)
XVI +	(1)	b	x = hvittingfoss
		d	7 = gaminara
		7	(d = gaminara)
		x	(b = hvittingfoss)
	(2)	k	2 = szentes
			z_{15} = orientalis
		2	(k = szentes)
		z_{15}	(k = orientalis)
XVII +		b	2 = kirkee
		2	(b = kirkee)
XVIII +		z_4	z_{23} = cerro
XXI +		b	x = minnesota
		x	(b = minnesota)
XXVIII +		y	7 = pomona
			z_{15} = telaviv
		7	(y = pomona)
		z_{15}	(y = telaviv)
XXX +		b	x = urbana
		x	(b = urbana)
XXXV +		f,g	adelaide
		m,t	monschaui
XXXVIII +		k	6 = inverness
		6	(k = inverness)
XXXIX +		k	5 = champaign
		5	(k = champaign)

N.B.: If the antigen was already listed in the same or in the last series, it appears in parentheses.

If a new salmonella type is recognized, the agglutination tests have to be repeated. Cross-absorption tests must be used to ascertain the quantitative relationship of the antigenic components. A new type should not be reported unless confirmed by another typing center.

The typing of salmonellas requires special experience, chiefly in the maintenance of stock cultures and in the preparation of serums. It is, therefore, advisable for the beginner to refrain from such work unless expert guidance is available.

REFERENCES

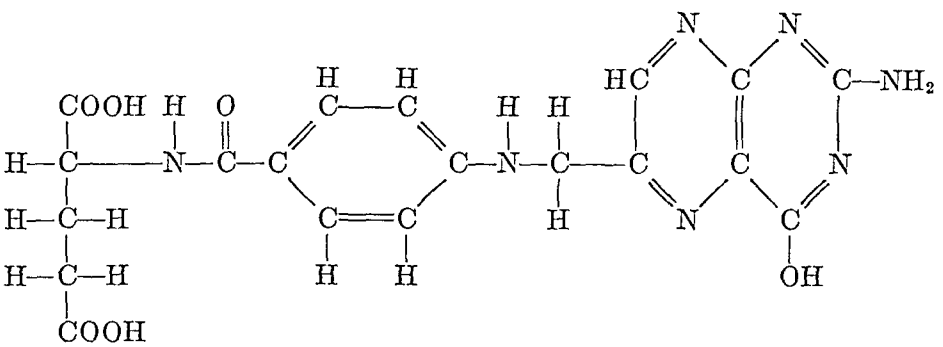
1. Crossley, V., Ferguson, M., Irvine, P., and Hastings, M.: Salmonella typing in Ontario and the use of polyvalent antisera. Canad. J. Pub. Health, **39**: 192–199, 1948.
2. Edwards, P. R., and Bruner, D. W.: Serological identification of Salmonella cultures. Kentucky Agr. Exper. Sta., Circular 54, 1943.
3. Edwards, P. R., and Bruner, D. W.: Notes on monophasic Salmonella cultures and their use in the production of diagnostic serums. J. Bact., **52**: 493–498, 1946.
4. Edwards, P. R., West, M. G., and Bruner, D. W.: Arizona group of paracolon bacteria. Kentucky Agr. Exper. Sta., Bulletin 499, 1947.
5. Ewing, W. H., and Bruner, D. W.: The use of a polyvalent serum for preliminary identification of Salmonella cultures. J. Bact., **53**: 362–363, 1947.
6. Felsenfeld, O., and Young, V. M.: A polyvalent serum for the diagnosis of Salmonella. J. Lab. and Clin. Med., **30**: 550–551, 1945.
7. Galton, M. M., and Quan, A. L.: Varieties of Salmonella isolated in Florida during 1942. Am. J. Hyg., **38**: 173–177, 1943.
8. Hajna, A. A.: Use of the "U" tube for the isolation of monophasic varieties from diphasic Salmonella cultures. J. Bact., **48**: 609–610, 1944.
9. Juenker, A. P.: A rapid method of phase isolation in Salmonella cultures. J. Bact., **52**: 609–610, 1946.
10. Kauffmann, F.: On the serology of the Salmonella group. Acta pathol., **24**: 242–249, 1947.
11. Kuhn, L. R.: Rapid method for producing suspensions of flagellar antigens and for inducing phase suppression. Am. J. Clin. Path., **17**: 569–570, 1947.
12. Seligmann, E., Saphra, I., and Wassermann, M.: Salmonella infections in the U. S. A. J. Immunol., **54**: 69–87, 1946.
13. Weil, A. J.: Personal communication to the author.

AMINOPTERIN (A FOLIC ACID ANTAGONIST) IN THE TREATMENT OF LEUKEMIA*

LEO M. MEYER, M.D., HAROLD FINK, M.D., ARTHUR SAWITSKY, M.D., MANUEL ROWEN, M.D., AND NORTON D. RITZ, M.D.

From the Medical Service, Kings County Hospital, Brooklyn, and Department of Therapeutics, New York University Medical College, New York, New York

Following the isolation and synthesis of pteroyl glutamic acid by SubbaRow and his co-workers[9] in 1946, chemical analogues and homologues having similar or antagonistic biologic activity have been developed. Synthetic folic acid, which is identical with the original *Lactobacillus casei* factor derived from liver has the following structural formula:



Pteroyl glutamic acid
(Folic acid)

A related compound having similar biologic activity, derived from molds, and recently synthesized, is pteroyl triglutamic acid (teropterin)[1].

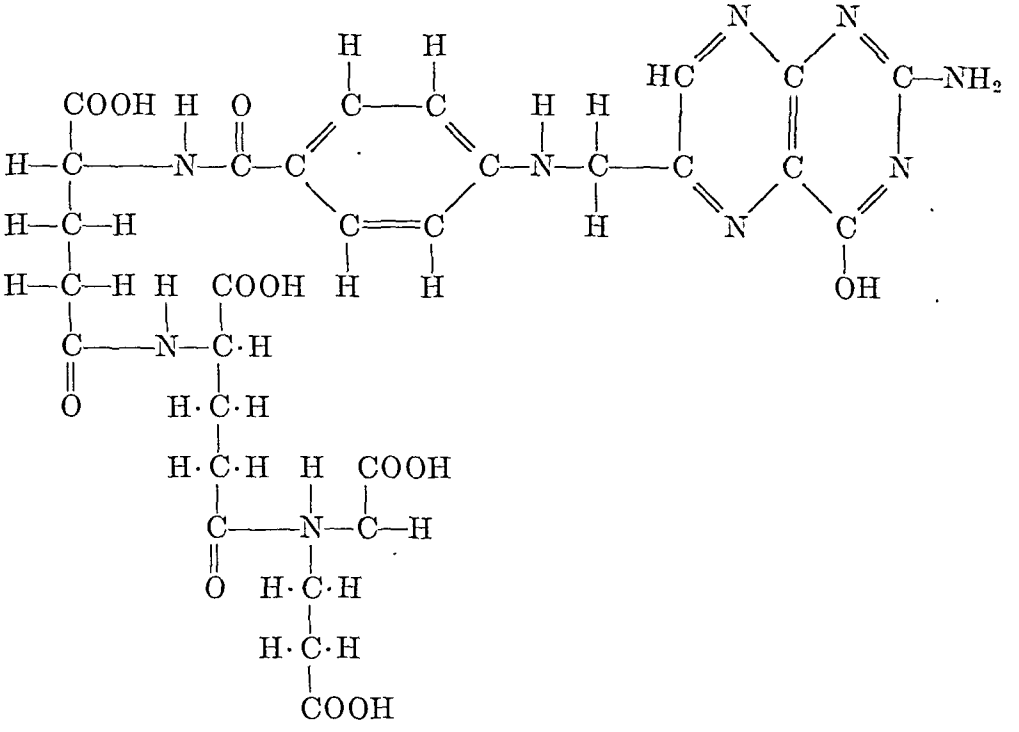
In 1944 Leuchtenberger *et al.*[3] demonstrated that pteroyl triglutamic acid (teropterin) inhibited the growth of transplanted sarcoma 180 in female Rockland mice and produced regression of spontaneous breast tumors in mice in 30 to 40 per cent of cases.[4] Pteroyl glutamic acid (folic acid) had no such effect.[5] Sugiura[10] was unable to confirm these experiments.

Little *et al.*[6] reported that Rous sarcoma failed to grow in chicks fed a folic acid deficient diet, whereas the addition of this compound produced neoplasm in 100 per cent of animals. They further demonstrated that inoculated chicks on a balanced diet containing folic acid failed to show any tumor growth when treated with three different folic acid antagonists, 1 of which was 4-amino pteroyl glutamic acid (aminopterin).

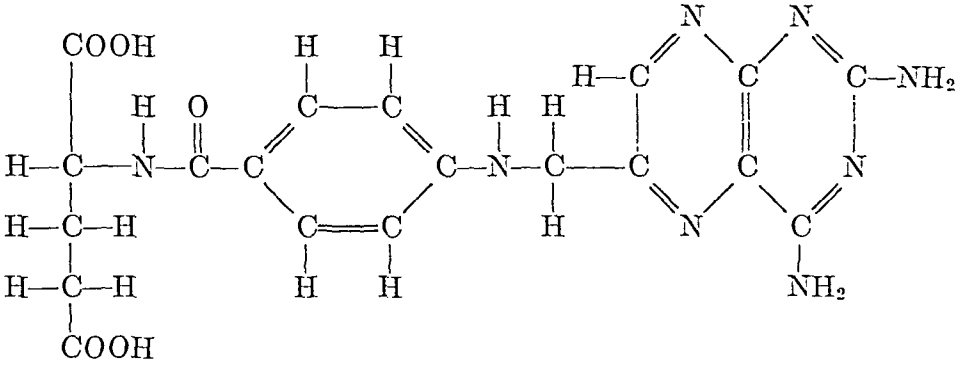
* Aided in part by a grant from the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York.

Received for publication, November 12, 1948.

In 1948, Meyer[7] reported temporary clinical and hematologic improvement in some patients with acute leukemia treated with the following folic acid antagonists, pteroyl aspartic acid (An-Fol-R) and methyl pteroic acid (Met-Fol-B).



Pteroyl triglutamic acid
(Teropterin)

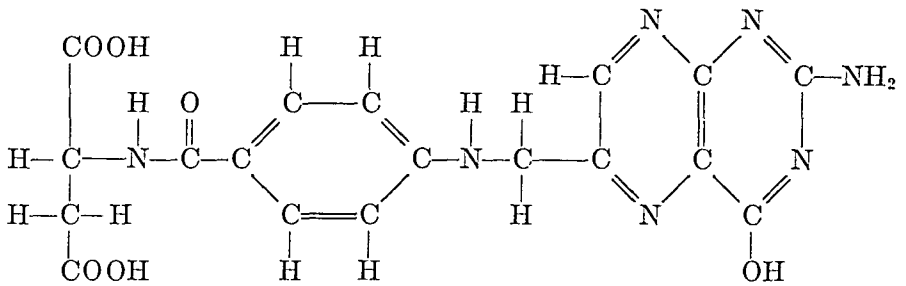


4-amino pteroyl glutamic acid
(Aminopterin)

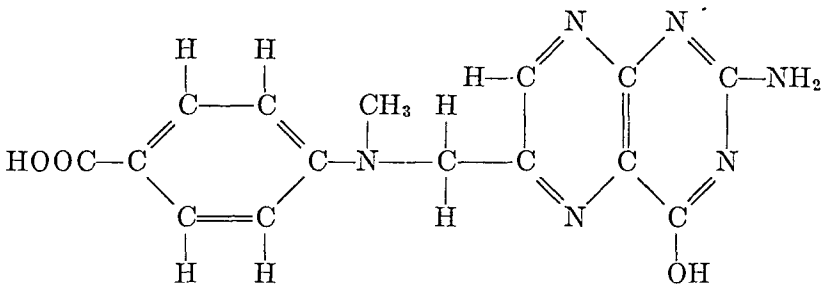
In a subsequent communication before the American Association for Cancer Research,[8] further studies with these 2 compounds and a third antagonist, 4-amino pteroyl glutamic acid (aminopterin) were presented. More recently, Farber *et al.*[2] have reported their experiences with aminopterin in 16 cases of

acute leukemia in children. These investigators reported clinical and hematologic improvement in 10 of their patients.

The present communication constitutes a summary of a collaborative study undertaken by the authors and several investigators in other institutions.* Detailed reports from each group are forthcoming.



Pteroyl aspartic acid
(An-Fol-R)



Methyl pteroic acid
(Met-Fol-B)

CLINICAL MATERIAL

Forty-three patients with leukemia were treated with aminopterin.** The leukemias were classified as follows:

21 myeloid
- in adults, 3 chronic, 14 acute
- in children, 4 acute

22 lymphoid
- in adults, 3 chronic, 7 acute
- in children, 12 acute.

* Dr. S. Estren, Mount Sinai Hospital, New York, N. Y.
Dr. D. K. Miller, University of Buffalo Medical College, Buffalo, N. Y.
Dr. F. M. Miller, Jefferson Medical College, Philadelphia, Pa.
Dr. A. A. Newman, New York Hospital, New York, N. Y.
Dr. C. H. Smith, New York Hospital, New York, N. Y.
Dr. S. O. Schwartz, Cook County Hospital, Chicago, Ill.

** Supplied by Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York.

Myeloid Leukemia

In adults. The 17 adults with myeloid leukemia ranged in age from 21 to 61 years. Five were men and 12 women. Chronic myeloid leukemia was diagnosed in 3, and acute myeloid leukemia in the remainder. Aminopterin was given intramuscularly in daily doses of from 0.5 to 5.0 mg. for periods of five days to four months, for a total of 2.5 to 600 mg. Two patients received teropterin concomitantly and 3 received liver extract. Other supportive therapy, penicillin, and whole blood transfusions were given as indicated. The patients remained under observation from 13 days to five months.

The anemia was unimproved with aminopterin therapy and the patients were supported by transfusions. The platelets were not affected. The white blood cells were unchanged in 6 patients. In 3 subjects there was an increase in cell count, while 1 patient with leukopenia remained leukopenic. In these 10 patients, the differential counts were unchanged. In the 7 patients who manifested high white cell counts, leukopenia developed in 5 and a normal cell count in the other 2. One of the latter showed no change in differential, while a reduction in the numbers of immature cells was noted in the other 6. In all of the patients studied, the bone marrow was hyperplastic and the predominant cell was the myeloblast. There was no change in the bone marrow in 14 of the patients treated. Two patients showed a reduction in numbers of cells but an unchanged differential pattern. Hypoplasia of the bone marrow developed in one patient, but the myeloblast remained the predominant cell.

Clinically, there was no apparent effect on the temperature, the lymph nodes, the spleen or the liver except in 2 of the patients in whom a reduction in the size of lymph nodes and spleen was noted.

Toxicity was of frequent occurrence. Alopecia, stomatitis, rectal bleeding, hematemesis, anal excoriation and severe vomiting were noted singly and in combination in 12 of the patients.

Evaluation of the clinical course shows that no beneficial clinical or hematologic effect was seen in 13 patients. There was a reduction in total numbers of white blood cells and immature forms in 4 patients, but severe toxicity prevented continuation of therapy in these cases.

In children. Four boys, 3 to 9 years of age, with acute myeloid leukemia were given intramuscular daily injections of 0.5 to 1.0 mg. of aminopterin for a total dose of 4 to 37 mg. Liver extract was administered with each dose to 3 of the children. The usual supportive therapy, penicillin and blood transfusions were utilized when indicated. The period of study ranged from eight to 37 days.

The anemia was unaffected except following blood transfusion. The platelet counts were unchanged. The peripheral blood, initially leukocytic, became leukopenic and myeloblasts disappeared from the peripheral blood of 3 patients. In the other child, the white cell count was reduced to normal levels, but the differential values were unchanged. In all patients the bone marrow, hyperplastic and myeloblastic, was not changed following therapy.

Aminopterin therapy had no effect on the fever noted in all subjects. Enlarged lymph nodes, liver and spleen were reduced in size in only 1 of the 4 pa-

tients. In 1 child, a second course of aminopterin therapy in higher dosage produced a temporary reduction in the size of enlarged lymph nodes and spleen.

Toxicity was not prominent. Hematemesis was observed in 1 boy given 0.5 mg. daily for 8 days.

An evaluation of the clinical course reveals no significant improvement in 3 children, and temporary but definite improvement in 1 subject.

Lymphoid Leukemia

In adults. Three patients with chronic lymphoid leukemia and 7 with acute lymphoid leukemia were treated with aminopterin. The ages of these patients varied from 29 to 67 years; 6 patients were men and 4 were women. Aminopterin was administered by daily intramuscular injection in doses of 0.5 to 2.0 mg. for a total of 2.5 to 51 mg. and the patients were observed for periods of 15 days to six months. General supportive therapy, penicillin and blood transfusions were given when necessary.

The anemia was helped only by blood transfusion. The platelets were initially reduced in number and not affected by therapy. In all 10 patients, there was no change in the differential counts of the peripheral blood. In 5 patients, the original leukopenia or leukocytosis was not influenced by the drug. Four patients with high white counts developed severe leukopenia with 1000 or fewer cells per cu. mm. One subject, originally leukopenic (2400 cells per cu. mm.), showed further cellular reduction (to 1100 cells per cu. mm.). The bone marrows of all the patients studied were hyperplastic and lymphoblastic. There was no change in the bone marrow of 7 patients, but a severe marrow hypoplasia was noted in the remaining 3.

Fever was reduced in 2 patients, but remained unaffected in 4 others. The size of the lymph nodes of 2 patients was temporarily reduced, but that of the liver and spleen was not affected.

Severe drug toxicity was observed in 4 subjects; a hemorrhagic skin rash appeared in 2, and stomatitis of moderate to severe degree was noted in 3; hematemesis was encountered in 1 person. In all these patients a marked leukopenia and bone marrow hypoplasia was observed.

Evaluation of the clinical course of this group indicates temporary improvement in only 1 patient, temporary reduction in lymph node enlargement in another and reduction of fever in a third.

In children. Aminopterin was administered to 12 children with acute lymphoid leukemia. There were 7 boys and 5 girls whose ages ranged from 2 to 13 years. Intramuscular aminopterin in doses of 0.25 to 1.0 mg. was injected daily. The total dosage per child varied from 3 mg. in a four-day period to 40 mg. over a period of 100 days. Liver extract was given with the aminopterin in 3 children. Penicillin, blood transfusions and other supportive therapy were used when indicated. These patients were observed from 10 days to 4 months.

Multiple transfusions helped support the anemia present in all. The platelet counts were not affected by aminopterin therapy. The peripheral blood counts showed initial leukocytosis in 7 patients, and in 5 of these, a subsequent leuko-

penia of 5000 to 1200. Normocytic levels were attained in 2 of the children. Three of the patients reverted to more normal appearing differential patterns for short periods of time; in 2 of these, lymphoblasts disappeared from the peripheral blood and a rise in segmented neutrophils was observed; in the other child, a significant fall in the numbers of lymphoblasts with concomitant rise in polymorphonuclear cells was seen. An initial leukopenia was noted in 5 children. Two became more severely leukopenic. The differential pattern did not change in any of these 5 subjects. Bone marrow aspirations were done on 11 patients, and in all 11, the bone marrows were hyperplastic and lymphoblastic. Aminopterin therapy did not influence the bone marrow of 5 children. Hypoplasia of marrow elements was noted in the other 6 and persisted even after the aminopterin therapy was discontinued. There was no significant alteration in the differential count of the bone marrow in any of the cases examined.

Following aminopterin therapy, the temperature curve of 2 patients showed good response, but that of 10 others was unaffected. Changes in size of lymph nodes, spleen and liver of the 12 children studied could not be predicted. In the majority, no effect was noted. In some, there was reduction in size of these organs, while in others further enlargement was seen.

Toxic manifestations to the drug were observed in 9 children. Stomatitis, hematemesis and rectal bleeding were the most common and most severe of the toxic signs. In 4 of the milder reactors, administration of the drug was continued without further progression of toxicity. In 4 others, therapy had to be discontinued because of the severity of these lesions. A marked persistent alopecia was encountered in 1 child, but aminopterin therapy was not discontinued. Severe purpura appeared in 2 patients terminally ill.

Evaluation of the clinical and hematologic course in this group indicated that 2 of the children studied showed temporary, but significant improvement. There was no notable effect on the clinical course of 8 of the patients. A severe persistent leukopenia with bone marrow hypoplasia was noted in one child, while in another, clinical and hematologic improvement followed cessation of aminopterin therapy.

SUMMARY OF FINDINGS AND DISCUSSION

Of 43 cases, 4 patients (9.3 per cent) showed improvement in hematologic and clinical pictures. The remainder showed no change or were adversely affected. The 4 improved patients were children, 3 of the acute lymphoid type and 1 of the acute myeloid type. No beneficial results were seen in adults. Patients with lymphoid leukemia, to whom aminopterin was administered, responded more rapidly and precipitously with peripheral leukopenia and bone marrow hypoplasia than those in the myeloid group.

Aminopterin had no beneficial effect on the anemia present in 41 patients, but 2 did show improvement in hemoglobin and red blood cell levels during treatment.

The effect of the drug on the white blood cells was variable. In 18 subjects

the leukocyte count was increased or unchanged, in 17 moderate leukopenia developed and in 4, severe leukopenia. In all these patients differential values were not changed with treatment. Hematologic improvement with reduction of cell count and maturity of cell type was observed in 4 patients.

Aminopterin appeared to have no effect on the blood platelet count.

Depression of the bone marrow was severe at times. In 2 patients there was moderate reduction in cellularity, while in 9 severe irreversible hypoplasia occurred. There was no detectable change in the bone marrow of 29 subjects.

Of 28 febrile patients, 4 showed reduction in temperature following treatment.

Enlarged lymph nodes were found in 25 patients. Of these, there was reduction in size in 6, no change in 15 and progressive enlargement in 4.

Hepatomegaly was encountered in 23 persons; 3 showed reduction in size, 1 an increase, and 19 no change. Splenomegaly was found in 28 subjects, 6 showed reduction in size of the spleen, 2 an increase and 20 no change.

Loss of hair occurred in 3 patients. Hemorrhage into the skin, associated with severe leukopenia and hypoplastic bone marrow, was noted in 2 others. Stomatitis was observed in 12 patients, hematemesis in 6, and rectal bleeding in 7.

The toxicity of aminopterin deserves emphasis. Leukopenia with severe irreversible hypoplasia of the marrow was the most serious complication. This toxic effect was more common in those patients receiving the drug for longer periods of time. Total dosage and dosage per body weight could not be related to marrow hypoplasia. Daily blood counts must be made and patients followed carefully since a patient with leukocytosis of 70,000 to 100,000 cells per cu. mm. may suddenly become severely leukopenic within a period of 24 to 72 hours. Mucous membrane lesions were the most frequent and most troublesome of the toxic signs. Shallow ulceration of buccal and pharnyngeal mucosa with the formation of an adherent dirty gray overlying membrane on an erythematous base was commonly seen. Hematemesis and rectal bleeding were also noted and were believed to be expressions of similar mucosal lesions within the gastrointestinal tract. The mucous membrane lesions occurred haphazardly and could not be related to dosage or duration of medication. Liver extract was not beneficial. In a few patients with lesions appearing early in the course of therapy, and of a mild nature, aminopterin was continued and no further progression of these lesions was noted. When the lesions were of serious nature withdrawal of the drug led rapidly to mucous membrane healing. Alopecia was occasionally seen. This complication was not considered cause for cessation of therapy. A maculo-papular hemorrhagic skin rash was sometimes encountered. The rash usually accompanied severe aminopterin toxicity with leukopenia, marrow hypoplasia and mucosal lesions.

The most striking findings in this study were the unpredictability of the action of aminopterin and the very evident toxicity of the drug. Both beneficial and toxic effects could not be controlled. Nor could these effects be related to dosage of aminopterin alone or to its administration simultaneously with liver extract, teropterin or other supportive measure.

CONCLUSIONS

1. The administration of aminopterin to 43 patients with leukemia was followed by changes in the peripheral blood and bone marrow in 18. In 4 persons a distinct improvement in hematologic findings was noted, while 14 developed moderate to severe leukopenia with hypoplasia of the bone marrow.

2. Aminopterin had no significant effect on the hemoglobin, erythrocytes, platelets or temperature of patients with acute leukemia.

3. The white blood cells and bone marrow of patients with lymphoblastic leukemia were more susceptible to the drug, but leukopenia and irreversible hypoplasia of the marrow occurred in both myeloid and lymphoid groups.

4. Four patients were clinically and hematologically improved, 15 showed toxic effects of the drug necessitating discontinuance of treatment, and in the remaining 24, the clinical course was unaffected.

5. Beneficial and toxic effects could not be related to dosage according to weight or to the simultaneous administration of liver extract or teropterin.

6. The unpredictable action of aminopterin and its evident toxicity indicate that this drug will have very limited use in the treatment of leukemia. Other related less toxic compounds may be of greater value.

REFERENCES

1. Boothe, J. H., Mowat, J. H., Hutchings, B. L., Angier, R. B., Waller, C. W., Stokstad, E. L. R., Semb, J., Gazzola, A. L., and SubbaRow, Y.: Synthesis of pteroyl tri-glutamic acid (teropterin). Trans. New York Acad. Sci., Special number, series 2, **10**: 70–71, 1948.
2. Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., and Wolff, J. A.: Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-amino pteroyl glutamic acid (aminopterin). New England J. Med., **238**: 787–793, 1948.
3. Leuchtenberger, C., Lewisohn, R., Lazlo, D., and Leuchtenberger, R.: "Folic acid", a tumor growth inhibitor. Proc. Soc. Exper. Biol. and Med., **55**: 204–205, 1944.
4. Leuchtenberger, R., Leuchtenberger, C., Lazlo, D., and Lewisohn, R.: The influence of "folic acid" on spontaneous breast cancers in mice. Science, **101**: 46, 1945.
5. Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., and Keresztesy, J. C.: The influence of liver *L. casei* factor on spontaneous breast cancer in mice. Science, **104**: 436–437, 1946.
6. Little, P. A., Sampath, A., Paganelli, V., Locke, E., and SubbaRow, Y.: The effect of folic acid and its antagonists on Rous chicken sarcoma. Trans. New York Acad. Sci., Special number, series 2, **10**: 91–98, 1948.
7. Meyer, L. M.: Use of folic acid derivatives in the treatment of human leukemia. Trans. New York Acad. Sci., Special number, series 2, **10**: 99–102, 1948.
8. Meyer, L. M., Fink, H., Ritz, N. D., Rowen, M. J., and Sawitsky, A.: Folic acid derivatives in the treatment of human leukemia. Proc. Am. Assn. Cancer Res., in print.
9. SubbaRow, Y. and others: Folic acid. Ann. New York Acad. Sc., **48**: 255–349, 1946.
10. Sugiura, K.: Effect of intravenous injection of yeast, barley extracts and *L. casei* factor upon spontaneous mammary carcinoma. Approaches to tumor chemotherapy. Am. Assn. Adv. Sc., 208–213, 1945.

USE OF A FOLIC ACID ANTAGONIST IN CHRONIC LEUKEMIA*

LAWRENCE BERMAN, M.D., ARNOLD R. AXELROD, M.D., ELMORE C. VONDER HEIDE, M.D., AND ELWOOD A. SHARP, M.D.

From the Departments of Pathology and Medicine, Wayne University College of Medicine, and City of Detroit Receiving Hospital, Detroit, Michigan

Synthetic substances capable of antagonizing the action of folic acid have been described recently.[6, 8, 11, 13] The materials have been tested on swine,[1, 5, 14] rats,[3] mice,[4] chicks,[3, 4, 7, 15] and various micro-organisms. The accelerating action of folic acid in acute leukemia led to the clinical use of folic acid antagonists.[2] Previous reports[2, 9, 10, 12] have dealt primarily with the treatment of acute leukemia.

This study is based on observations of the effect of the folic acid antagonist, 4-amino pteroylglutamic acid (N-[4-{[(2,4-diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid) in nine patients with chronic leukemia. One of the patients having myelogenous leukemia received two series of treatments. The drug was taken orally, in daily doses ranging from 1.25 to 7.5 mg. over periods from 6 to 25 days. Results are shown in Tables 1 and 2, and in Charts 1 through 4.

DISCUSSION

Farber and co-workers[2] used 4-amino pteroylglutamic acid, known commercially as aminopterin, in 16 children with acute leukemia. In 10 they observed temporary clinical and hematologic improvement. The leukocyte counts returned to normal, whether they were initially high or low. Immature cells were reduced in number and stem cells often disappeared entirely. In most cases the enlarged livers, spleens and lymph nodes became normal in dimensions. Similar but less striking results have been reported by others.[10, 12] Meyer[9] observed no hematologic response following the use of folic acid antagonists in 3 patients with chronic lymphatic leukemia. Judging from the comparison which can be made from observations on acute leukemia, in which numerous stem cells are present in the blood, and Meyer's 3 cases of chronic lymphatic leukemia, in which condition few, if any, stem cells, are found, it seems evident that stem cells and other immature leukocytes are more sensitive to the folic acid antagonists than are the more differentiated cells.

In our study we have observed that stem cells in both myelogenous and lymphatic leukemia, and immature granulocytes in myelogenous leukemia decreased in number, but platelet and erythrocyte counts were variable. Usually mature granulocytes decreased in number during treatment, but lymphocytes of mature type were relatively unaffected. These observations indicate that, in addition to the morphologic similarity of lymphoblasts and myeloblasts, there are also

* Read by title at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 15, 1948.

Received for publication, November 13, 1948.

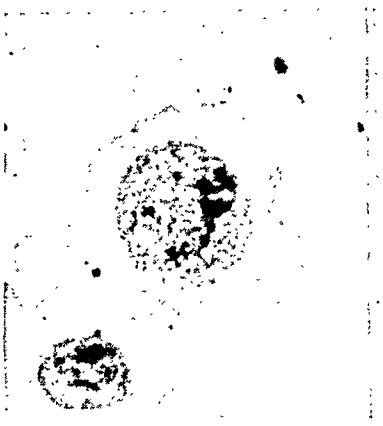


FIG. 1

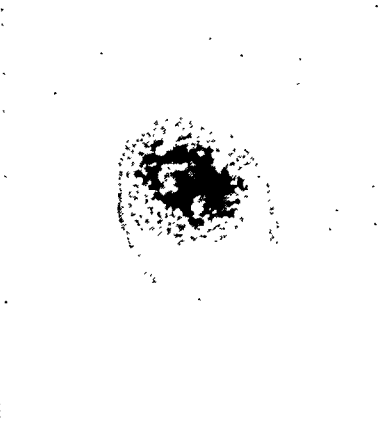


FIG. 2

FIG. 1. Basophilic megaloblast in bone marrow smear from a patient with chronic myelogenous leukemia after treatment with 4-amino pteroylglutamic acid.

FIG. 2. Basophilic megaloblast in bone marrow smear from a patient with chronic lymphatic leukemia after treatment with 4-amino pteroylglutamic acid.

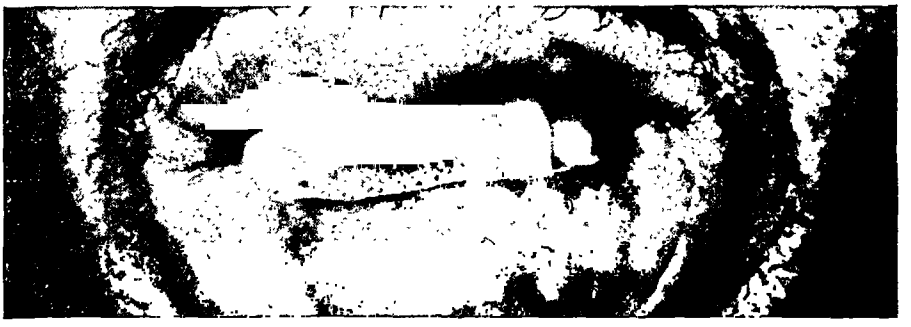


FIG. 3. Superficial necrosis of oral mucosa typical of early changes seen in patients receiving 4-amino pteroylglutamic acid.

TABLE 1

LEUKOCYTE COUNTS IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA BEFORE AND AFTER TREATMENT

CASE NO.	TOTAL WBC PER CU. MM.	STEM CELLS PER CU. MM.	IMMATURE GRANULOCYTES PER CU. MM.	MATURE GRANULOCYTES PER CU. MM.	TOTAL DOSE IN MG.
1a	300,000	14,280	121,200	109,000	0
	15,000	0	856	7,450	117
1b	100,000	4,330	22,080	62,400	0
	68,000	302	15,410	37,200	49
2	150,000	7,810	72,000	49,500	0
	28,700	287	861	20,900	97
3	47,800	8,560	10,710	24,580	0
	18,850	296	1,059	14,320	97
4	263,000	27,920	98,600	100,800	0
	184,000	6,930	5,550	118,800	86
5	78,000	19,080	8,860	33,300	0
	5,750	39	78	3,921	67

TABLE 2

LEUKOCYTE COUNTS IN PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA BEFORE AND AFTER TREATMENT

CASE NO.	TOTAL WBC PER CU. MM.	STEM CELLS PER CU. MM.	GRANULOCYTES AND MONOCYTES	LYMPHOCYTES	TOTAL DOSE IN MG.
1	102,000	4,270	15,500	82,200	0
	73,800	5,740	260	67,800	132
2	76,400	0	12,500	63,900	0
	77,800	0	8,600	69,200	105
3	35,130	0	4,567	30,563	0
	50,000	0	10,500	39,500	52
4	231,000	26,240	6,644	198,100	0
	180,500	8,570	7,876	164,000	56

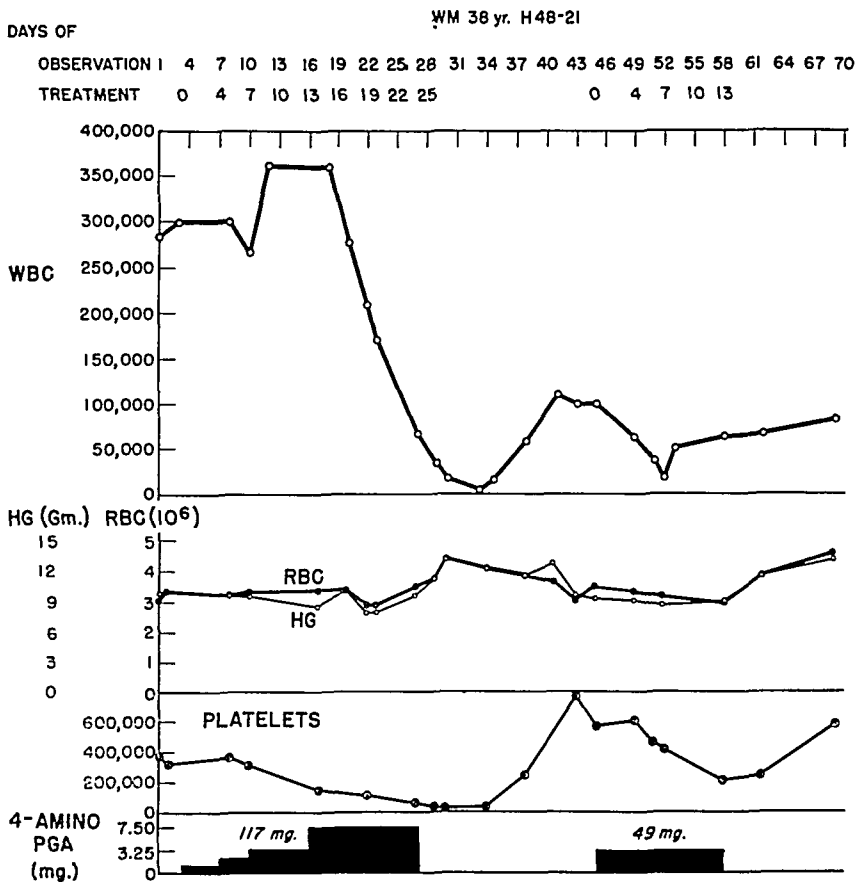


CHART 1. Hematologic data of Case 1a and 1b, chronic myelogenous leukemia

functional similarities in the response to folic acid antagonists. Progressive falls in total leukocyte counts occurred for as long as 10 days after treatment was stopped. The most important morphologic effects included the appearance of

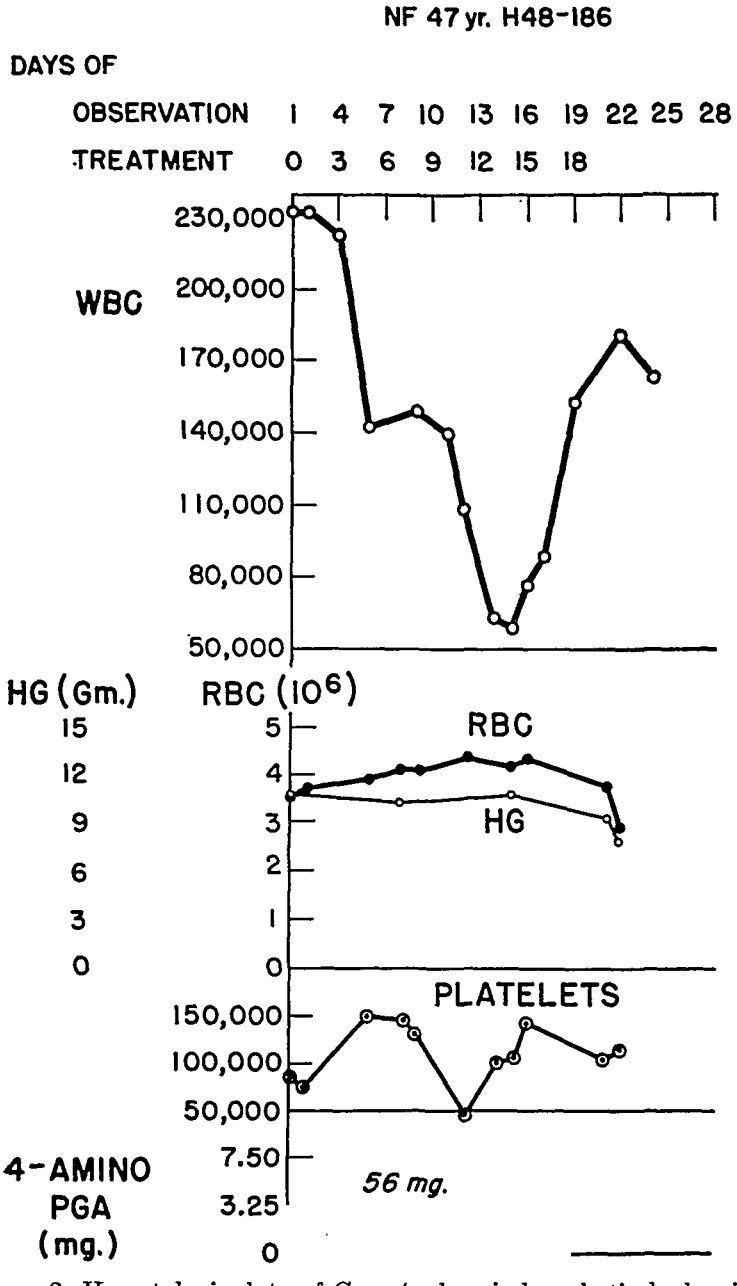


CHART 2. Hematologic data of Case 4, chronic lymphatic leukemia

small numbers of megaloblasts in material aspirated from the sternal marrow. The cells were indistinguishable from those characterizing erythropoiesis in Addisonian pernicious anemia in relapse (Figs. 1 and 2). Usually they were seen after approximately 50 to 100 mg. of the medication had been ingested over periods of from 7 to 23 days.

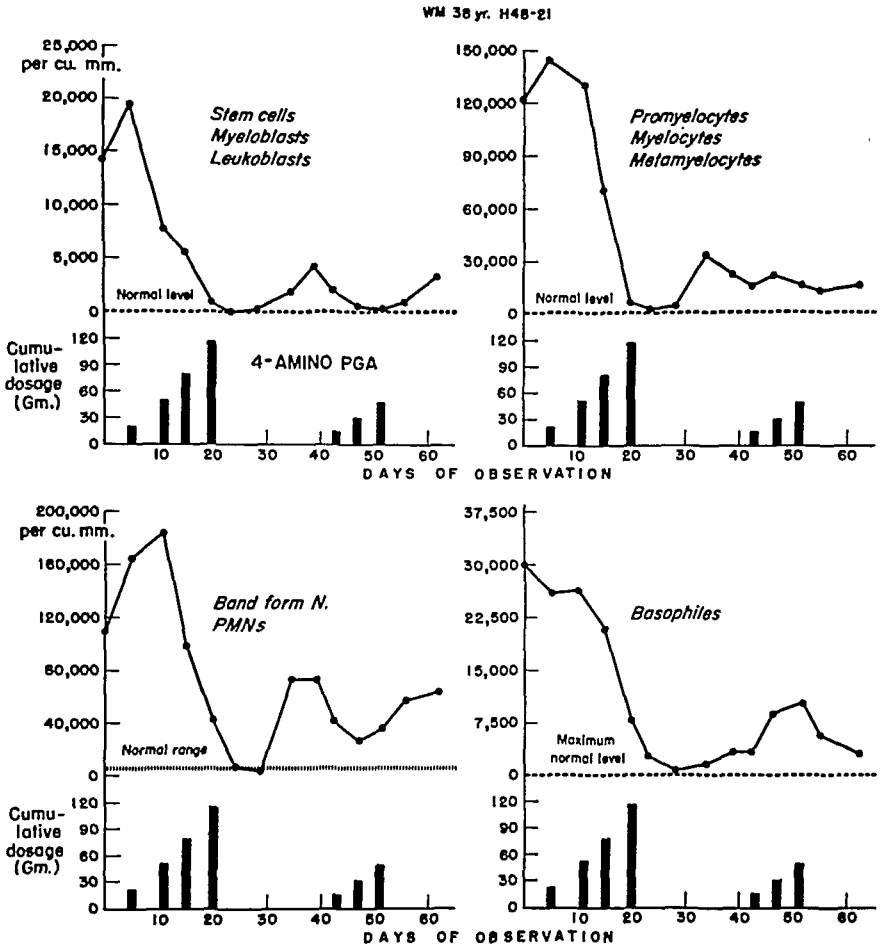


CHART 3. Absolute counts of undifferentiated cells, immature granulocytes, and mature granulocytes. Case 1a and 1b, chronic myelogenous leukemia.

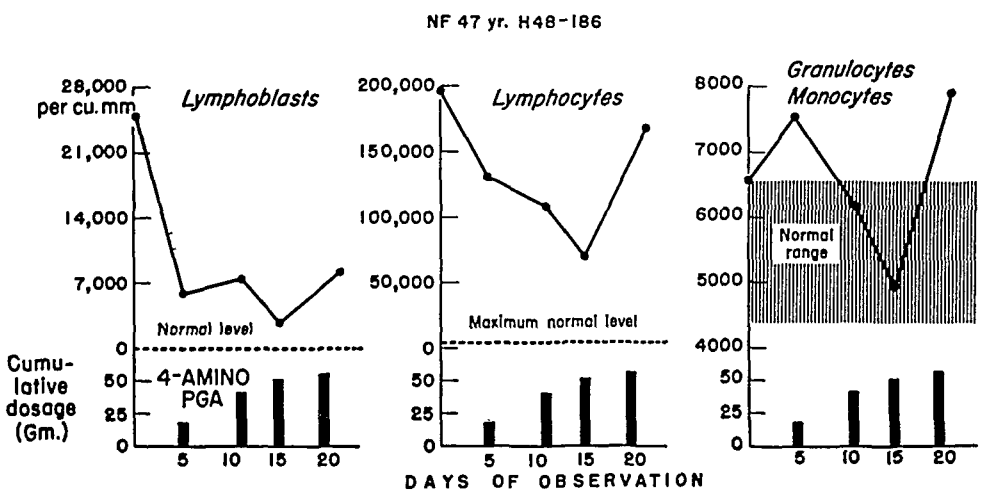


CHART 4. Absolute counts of lymphoblasts, mature lymphocytes, granulocytes and monocytes. Case 4, chronic lymphatic leukemia.

In 2 patients with lymphatic leukemia there was a decrease in the sizes of lymph nodes. The hematologic effects of therapy were temporary and never completely adequate because treatment had to be stopped when toxic signs appeared.

The toxic effects, in the order of appearance, included pharyngitis, stomatitis, sore tongue, crampy abdominal pain sometimes associated with diarrhea, dermatitis, and alopecia (Fig. 3). Usually pharyngitis first appeared within a few days before or after significant changes in the leukocyte counts were noted. The total dosage of the drug which produced toxic effects varied from 30 to 75 mg., although one patient received 105 mg. without experiencing such signs. There was evidence that when small doses are used initially, a larger cumulative dose can be tolerated before toxic symptoms appear. Autopsies of two patients, who expired shortly after therapy was stopped, revealed no changes which could be attributed to the medication.

SUMMARY

The clinical and hematologic effects of 4-amino pteroylglutamic acid therapy in the treatment of 9 patients with chronic leukemia are reported.

Stem cells, granulocytes and immature lymphocytes decreased in number during treatment, but mature lymphocytes were relatively unaffected.

There was no regular effect on the erythrocyte and platelet counts. There was a disturbance of erythropoiesis characterized by the appearance of megaloblasts in the marrow of treated patients, reflecting folic acid deficiency produced by the use of the folic acid antagonist.

Although 4-amino pteroylglutamic acid has a definite hematologic effect, there was no subjective improvement in any of the patients.

The use of the drug in chronic leukemia is limited by the early development of toxic phenomena.

Acknowledgments. The technical assistance for this study was furnished by the Anemia Laboratory, Out-patient Department, Harper Hospital, Detroit. The photomicrographs were made by Charles Pickard and Lester Walter.

REFERENCES

1. Cartwright, G. E., Fay, Jane, Tatting, Betty, and Wintrobe, M. M.: Pteroylglutamic acid deficiency in swine; effects of treatment with pteroylglutamic acid, liver extract, and protein. J. Lab. and Clin. Med., **33:** 397–416, 1948.
2. Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., and Wolff, J. A.: Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. New England J. Med., **238:** 787–793, 1948.
3. Franklin, A. L., Stokstad, E. L. R., Belt, Margaret, and Jukes, T. H.: Biochemical experiments with a synthetic preparation having action antagonistic to that of pteroylglutamic acid. J. Biol. Chem., **169:** 427–435, 1947.
4. Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H.: Acceleration of pteroylglutamic acid deficiency in mice and chicks by a chemical antagonist. Proc. Soc. Exper. Biol. and Med., **65:** 368–370, 1947.
5. Heinle, R. W., Welch, A. D., George, W. L., Epstein, M., and Pritchard, J. A.: Effect of extrinsic factor, liver extract, and folic acid on induced macrocytic anemia of swine. Proc. Central Soc. Clin. Research, **20:** 7, 1947.
6. Hutchings, B. L., Mowat, J. H., Oleson, J. J., Stokstad, E. L. R., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y.: Pteroylaspartic acid, an antagonist for pteroylglutamic acid. J. Biol. Chem., **170:** 323–328, 1947.
7. Little, P. A., Sampath, A., Pagenelli, E. L., and SubbaRow, Y.: The effect of folic acid and its antagonists on Rous chicken sarcoma. Trans. New York Acad. Sci., Special number, series 2, **10:** 91–98, 1948.

8. MARTIN, G. J., TOLMAN, L., AND MOSS, J.: d—methylfolic acid displacing agent for folic acid. Arch. Biochem., **12:** 318–319, 1947.
9. MEYER, L. M.: Use of folic acid derivatives in treatment of human leukemia. Trans. New York Acad. Sci., Special number, series 2, **10:** 99–102, 1948.
10. PIERCE, MILA, AND ALT, H.: Treatment of acute leukemia with aminopterin (4-aminopteroylglutamic acid). Proc. Central Soc. Clin. Research, **21:** 89–90, 1948.
11. SMITH, J. M., Jr., COUSULICH, DONNA B., HULTQUIST, M. E., AND SEEGER, DORIS, R.: The chemistry of certain pteroylglutamic acid antagonists. Trans. New York Acad. Sci., Special number, series 2, **10:** 82–83, 1948.
12. STICKNEY, J. M., HAGEDORN, A. B., MILLS, S. D., AND COOPER, T.: Changes in blood and bone marrow in acute leukemia induced by aminopterin. Proc. Central Soc. Clin. Research, **21:** 31, 1948.
13. SWENSEID, M. E., WITTLE, E. L., MOERSCH, G. W., BIRD, O. D., AND BROWN, R. A.: Studies in the rat of inhibitors of pteroylglutamic acid structurally related to this vitamin. Proc. Federation of American Soc. Exper. Biol., **7:** 299–300, 1948.
14. WELCH, A. D., HEINLE, R. W., SHARPE, G., GEORGE, W. L., AND EPSTEIN, M.: Chemical antagonists of pteroylglutamic acid in a pig; hematopoietic effect of extrinsic and intrinsic factors. Proc. Soc. Exper. Biol. and Med., **65:** 364–368, 1947.
15. WOLL, E.: The Rous sarcoma in birds treated with folic acid and its derivatives; a pathologic study. Trans. New York Acad. Sci., Special number, series 2, **10:** 83–91, 1948.

FURTHER EVIDENCE FOR ANTIBODIES OF THIRD ORDER

Fractionation of Agglutinins, Blocking Antibodies and Cryptagglutinoids by Physicochemical Methods*

JOSEPH M. HILL, M.D., SOL HABERMAN, Ph.D., AND RUTH GUY, B.A.

From the William Buchanan Blood Center, Baylor Hospital and the Department of Clinical Pathology, Southwestern Medical College, Dallas, Texas

In 1946 a third variety or order of reactivity of Rh antibodies was first reported by the present authors.[3, 5, 6] These antibodies, later termed cryptagglutinoids,[4, 7, 8] were shown to differ from the classical agglutinin and the blocking antibody of Wiener[12] and Race[9] in that they neither agglutinated saline suspensions of red cells nor gave positive blocking tests. Originally these cryptagglutinoids were detected by the Coombs (developing) test[1] and/or by quantitative studies of hemolytic activity.[4, 6, 7, 8] When the albumin agglutination test of Diamond and Denton[2] and the serum suspension method of Wiener[13] were employed, it was found that almost all cryptagglutinoid antibodies could be detected and titrated by these technics.

Comparison of the Rh antibody titers of various serums obtained by the developing test, blocking test, the albumin test and the serum test revealed antibody patterns inconsistent with the concept that the latter two technics were merely more sensitive methods for detection of blocking antibodies. For example, in a previous report[7] cryptagglutinoid titers as high as 1/32,000 by developing test were found without evidence of either "saline" agglutinins or antibodies demonstrable by Wiener's blocking test. The albumin technic gave titers equivalent to those of the developing test when applied to such serums.

After the accumulation of extensive data from studies of antibody patterns by multiple tests, it was found[8] that Rh antibodies could be characterized serologically as shown in Table 1. The variety of reactions found suggested that there might be detectable chemical differences in Rh antibodies which could be exploited to characterize further and possibly separate the different varieties. The first efforts[7] to separate cryptagglutinoids from saline agglutinins by electrophoretic methods were inconclusive but showed antibody activity in the beta as well as in the gamma globulin fractions. In the same year, Witebsky *et al.*[14] had reported the separation of a blocking antibody from "saline" agglutinin by a simple dialysis technic. The blocking antibody was found mainly in the supernatant (albumin).

With further dialysis studies, Witebsky[15] in 1948 confirmed the existence of Rh antibodies other than saline agglutinins and blocking antibodies. Use of the dialysis method in our laboratory gave similar results. However, because

* Presented at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 14, 1948.
Aided by a grant from Mrs. E. Wadley Moseley.
Received for publication, October 14, 1948.

of the uncertain control of salt concentration and pH, a more precise method of fractionation with minimal protein denaturation was sought; cold alcohol fractionation had been found to give considerable loss of titer apparently through denaturation. Electrophoretic separation had the disadvantage of dilution and small yield for the amount of technical difficulties. The new Reid and Jones[10, 11] plasma protein fractionation process, employing ion-exchange resins, proved to be especially suitable for antibody separation.

METHOD

The globulins of Rh antibody-containing serums were precipitated through the use of ion-exchange resins as described by Reid and Jones[10, 11]. The globulin

TABLE 1

ANTIBODY DESIGNATION	TESTS CHARACTERIZING HETEROGENEITY OF CDE-cde ANTIBODIES					
	Agglutination in Saline	Blocking Test (Wiener)	Capillary (Chown)	Albumin Aggt. (Diamond)	Developing Test (Coombs)	Hemolysis (Hill and Haberman)
Classical agglutinin	+	−	+	+	+	+
Blocking antibody (agglutinoid)	−	+	−	+	+	+
"Cryptagglutinoid" (3rd order antibody)	−	−	+	+	+	+
	−	−	−	+	+	+
	−	−	−	−	+	+

sub-fractions desired were obtained by dissolving the precipitate from 50 ml. of original serum in selected molar concentrations of sodium chloride. Each such fraction was made up to the original volume of 50 ml. All fractions were related to the albumin and the alpha, beta and gamma globulin components of human serum by electrophoretic studies. The following serologic technics were employed: the tube saline agglutination test, the blocking test as described by Wiener,[12] the albumin test of Diamond and Denton[2] and the anti-globulin technic of Coombs *et al.*[1] as used in this laboratory.[3] Insofar as possible, titrations were performed immediately after fractionation, since all antibodies were found to be quite labile in saline solution after separation.

RESULTS

Fractionation of serums containing Rh antibodies by the Reid process gave results as illustrated in Tables 2 and 3. Both serums in their original state appeared devoid of classical agglutinins with respect to antigen D when tested against saline suspensions of appropriate Rh-positive erythrocytes but showed the relatively strong blocking titer of 1/128 and the exceptionally high cryptagglutinoid titer of 1/128,000 and 1/512,000 respectively. The first precipitation

by ion-exchange resins yielded two fractions: (1) the albumin- and water-soluble globulin (euglobulin) which contained most of the blocking titer and approxi-

TABLE 2

FRACTIONATION OF ANTI-CD SERUM

SERUM SAMPLE I

	ANTIGEN D IN RED BLOOD CELLS TESTED			ANTIGEN C IN RED BLOOD CELLS TESTED		
	Saline Agglutination	Blocking Test	Coombs' Test and/or Albumin	Saline Agglutination	Blocking Test	Coombs' Test and/or Albumin
Whole serum	—	1/128	1/128,000	1/256	—	1/1000
Total globulin	1/512	—	1/64,000	1/512	—	1/1000
Albumin and euglobulin	—	1/192	1/96,000	—	—	1/192
Albumin	—	—	—	—	—	—
Euglobulin	—	1/8	1/512	—	—	1/8±
0–.001 M NaCl	—	1/8	1/64	—	—	—
.001–.01 " "	—	1/4	1/512	—	—	—
.01–.025 " "	—	1/8	1/8000	1/1	—	1/1
.025–.05 " "	1/4	1/8	1/8000	1/32	—	1/16
.05–.075 " "	1/64	—	1/1000	1/64	—	1/32
.075–.1 " "	1/64	—	1/64	1/64	—	1/32
.1–.115 " "	1/64	—	1/16	1/64	—	1/16
.115–.15 " "	1/64	—	1/8	1/64	—	1/8

TABLE 3

FRACTIONATION OF ANTI-CD SERUM

SERUM SAMPLE II

	ANTIGEN D IN RED BLOOD CELLS TESTED			ANTIGEN C IN RED BLOOD CELLS TESTED		
	Saline Agglutination	Blocking Test	Coombs' Test and/or Albumin	Saline Agglutination	Blocking Test	Coombs' Test and/or Albumin
Whole serum	—	1/128	1/512,000	1/512	—	1/512
Total globulin	1/128	—	1/1000	1/128	—	1/32
Albumin and euglobulin	—	1/8	1/512	—	—	—
Albumin	—	—	—	—	—	—
Euglobulin	—	1/32	1/256	—	—	—
.001 M NaCl	—	—	—	—	—	—
.005 " "	—	—	1/32	1/1	—	1/1±
.02 " "	1/2±	1/8	1/4±	1/16	—	1/4
.03 " "	1/2	1/1	1/256	1/16	—	1/8
.05 " "	1/2	1/2	1/1000	1/4	—	1/1
.1 " "	1/64	—	1/16	1/64	—	1/32
.2 " "	1/64	—	1/128	1/32	—	1/4

mately one-half of the cryptagglutinoids, and (2) the precipitated alpha, beta and gamma globulins which grossly appeared to contain cryptagglutinoids and heretofore undetected agglutinins but no blocking antibodies. A further fractionation of the euglobulin from the albumin by sodium sulfate precipitation

showed that the blocking antibody remained completely with the euglobulin, whereas the albumin remained devoid of antibodies.

The further fractionation of the total globulin precipitated by differential solubility in selected solutions of sodium chloride of different ionic strengths into seven fractions covered the range of alpha through gamma globulins. These fractions of serum sample I clearly showed that the "saline" agglutinin was limited to the gamma globulin subfractions. A weak blocking antibody titer which was not demonstrable in the total globulin, became apparent in the globulin subfractions in the alpha and beta zones. In serum sample I, the "saline" agglutinin and the blocking antibody appeared to be mutually exclusive through the alpha, beta, gamma range. The cryptagglutinoid of the globulin precipitate was distributed throughout all the subfractions except the gamma globulin but with a definite peak concentration in the beta globulin.

The anti-C component for serum sample I in the original state exhibited a "saline" agglutinin titer of 1/256, no blocking antibody and a titer in albumin of 1/1000. Fractionation showed the C agglutinin in the gamma globulin. No blocking antibody was demonstrated in any fraction. Substantially all of the weak cryptagglutinoid seemed to be isolated in the albumin-euglobulin fraction since the albumin titers in the beta and gamma globulin fractions were in each instance, except the .01M—.025M saline fraction, lower than the titers for the same fractions tested with saline suspensions of erythrocytes. Similarly, the fractionation of serum sample II with respect to the anti-C specificity antibodies showed consistently lower titers against albumin-suspended erythrocytes as compared with saline suspensions, not only for the beta and gamma globulin fractions, but for the total globulin as well. The C antibody in serum II was considered to be a pure (saline) agglutinin.

DISCUSSION

The quantitative expression of the data in Tables 2 and 3 by means of numerical titers must be considered with some caution. First it should be noted that there was apparently no loss of titer but a slight possible increase as a result of the first fractionation of serum I when we consider that both the total globulin and albumin-euglobulin fractions were made up to the original 50 ml. volume. On the other hand with serum II a great loss occurred at the same stage of fractionation. This loss, we believe, was due to the fact that the actual resin treatment, separation of precipitate and reconstitution in saline were drawn out over an excessive period of time. These two liquid fractions also were not immediately tested for antibody as in the first serum. Secondly, the sodium sulfate precipitation of euglobulin always resulted in loss of titer. The subfractionations which were promptly tested for antibody titers otherwise did not result in any substantial loss of antibody. Since each subfraction was made up in a volume of saline equal to the original volume of serum, the titers of the subfractions may be regarded as units of a given type of antibody and added together for comparison with antibody content of the total globulin fraction.

The results obtained in these experiments present evidence of physical chemi-

cal differences between varieties of Rh antibodies, identical in specificity, consistent with previously reported serologic characterizations as condensed in Table 1. Three varieties, or orders, of reactivity were defined: (1) the agglutinin which clumped erythrocytes in saline suspension, (2) the blocking antibody as originally defined,[12] and (3) the cryptagglutinoids which hemolyze in the presence of complement, adsorb specifically as shown by the Coombs developing test and in most, but not all, cases agglutinate erythrocytes suspended in albumin or serum. These cryptagglutinoids neither agglutinate red cells in saline suspensions nor do they give positive blocking tests.

The evidence presented in Tables 2 and 3 clearly demonstrates that the agglutinin active in saline is confined to the gamma globulin fraction in each serum examined. Although neither whole serum appeared to contain agglutinins against D, the removal of the albumin-euglobulin fraction revealed substantial

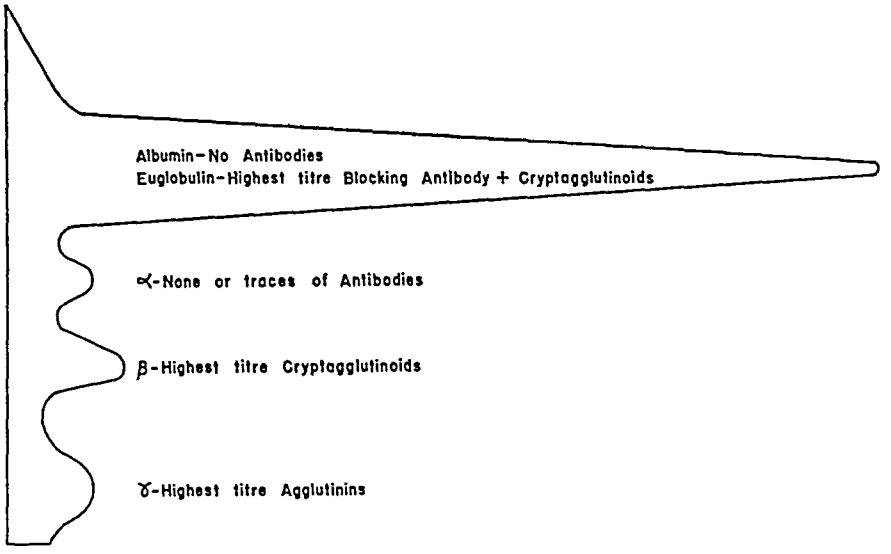


FIG. 1. Diagram of concentration of various antibodies with respect to electrophoretic pattern of serum

agglutinin titers of this specificity which had previously been masked by the blocking antibodies. The total globulin fractions (less the euglobulin) showed titers of 1/512 and 1/128 respectively with no blocking antibody apparent. However, these same globulin fractions when subfractionated revealed low titer-blocking antibodies in the low ionic strength fractions (.001 M—.025 M saline) while the agglutinins were found in the higher subfractions (.025 M—.15 M saline). Possibly the agglutinins and blocking antibodies are somewhat competitive with respect to the antigen.

The blocking antibody showed its greatest concentration in the euglobulin or combined albumin euglobulin fraction. Some blocking antibody titer was also found in the lower ionic strength subfractions in the alpha and beta globulins but in no case in the gamma globulin.

The cryptagglutinoids against D seemed to divide about equally between the albumin-euglobulin and the total globulin fractions in the case of both serums.

On further fractionation these antibodies are found in high titer in the euglobulin subfraction and in the mid-ionic strength subfractions of the beta globulin. It should be noted that the low titers by the Coombs or albumin technics in the gamma globulin subfractions (.075—.2 M saline) are interpreted as due to the activity of the agglutinin content rather than cryptagglutinoid. The titers shown seem to indicate that pure agglutinin is less active in albumin than in saline. If the cryptagglutinoid concentration is plotted over the electrophoretic pattern of the original serum we find a bimodal curve with peaks in the euglobulin and beta globulin. This suggests that cryptagglutinoids are not chemically homogeneous but appear to be made up of two main components. This is compatible with earlier serologic characterizations as shown in Table 1. However it would seem undesirable to subdivide further cryptagglutinoids until more is known of their mode of action or new and significant immunologic characteristics demonstrated.

In regard to the anti-C component, serum sample II, no antibody other than "saline" agglutinin was required to account for the data. Serum I, however, apparently contained a weak anti-C cryptagglutinoid substantially all of which may have gone with the euglobulin. This statement is made in view of the observation that marked loss of titer occurred with all sodium sulfate separations of the euglobulin and in accordance with the observed slightly lower titers with the albumin test in the subfractions from .01M to .2M saline, which seem due to "saline" agglutinin only.

In Figure 1 the location of the chief concentrations of the different varieties or orders of antibodies with respect to the electrophoretic pattern of serum containing anti-D Rh antibodies is given in simplified form.

SUMMARY

1. Separation of agglutinins, blocking antibodies, and cryptagglutinoids from anti-Rh (CD) human serum by physical chemical fractionation is described.

2. Further evidence of the heterogeneity of Rh antibodies of a given specificity (C + D) is presented.

3. These antibody fractions are further characterized by their serologic as well as electrophoretic patterns.

REFERENCES

1. Coombs, R. R. A., Mourant, A. E., and Race, R. R.: Detection of weak and "incomplete" Rh agglutinins; a new test. Brit. J. Exper. Path., **26**: 255–266, 1945; Lancet, **2**: 15–16, 1945.
2. Diamond, Louis K., and Denton, R. L.: Rh agglutination in various media with particular reference to the value of albumin. J. Lab. and Clin. Med., **30**: 821–830, 1945.
3. Haberman, S., and Hill, J. M.: Recent advances in Rh testing. Texas State J. M., **42**: 197–202, 1946.
4. Haberman, S., Hill, J. M., Everist, B. W., and Davenport, J. W., Jr.: The demonstration and characterization of the anti-d agglutinin and antigen predicted by Fisher and Race. Blood, **3**: 682–688, 1948.
5. Hill, J. M., and Haberman, S.: The Rh factor: analysis of a routine Rh testing service. Texas State J. M., **42**: 193–197, 1946.
6. Hill, J. M., and Haberman, S.: Demonstration of Rh antibodies in the new-born and further evidence of the pathogenesis of erythroblastosis. J. Lab. and Clin. Med., **31**: 1053–1066, 1946.

7. HILL, J. M., HABERMAN, S., AND JONES, FRANCES: Hemolytic Rh immune globulins: evidence for a possible third order of antibodies incapable of agglutination or blocking. Blood (Suppl. 2), **3**: 80-99, 1948.
8. HILL, J. M., HABERMAN, S., AND MUIRHEAD, E. E.: CDE-cde (Rh-Hr) blood antigens and antibodies. Scientific Exhibit, 96th Annual Session, American Medical Assn., June 1947, Atlantic City, New Jersey.
9. RACE, R. R., AND TAYLOR, G. L.: Incomplete antibody in Rh immunization. Brit. M. J., **2**: 756, 1944.
10. REID, ALLEN, AND JONES, FRANCES: Method for fractionation of plasma proteins for therapeutic use. Read at the First Congress of the International Society of Hematology, Buffalo, New York, August, 1948.
11. REID, ALLEN F., AND JONES, FRANCES: Production of therapeutic fractions of human blood serum by removal of salts with ion-exchange resins. Am. J. Clin. Path., **19**: 10-15, 1949.
12. WIENER, A. S.: A new test (blocking test) for Rh sensitization. Proc. Soc. Exper. Biol. and Med., **56**: 173-176, 1944.
13. WIENER, A. S.: Conglutination test for Rh sensitization. J. Lab. and Clin. Med., **30**: 662-667, 1945.
14. WITEBSKY, E., MOHN, J. F., HOWLES, D. J., AND WARD, H. M.: A simple method for the concentration of Rh agglutinins. Proc. Soc. Exper. Biol. and Med., **61**: 1-5, 1946.
15. WITEBSKY, E.: Cited in report of the Advisory Review Board on the Nomenclature of the Anti-Rh Typing Serums. Science, **107**: 27-31, 1948.

A METHOD FOR THE QUANTITATIVE MEASUREMENT OF COMPLEMENT-FIXATION*

E. E. ECKER, PH.D., AND C. W. HIATT, PH.D.

From the Institute of Pathology, Western Reserve University, and the University Hospitals, Cleveland, Ohio

Sensitized erythrocytes are subject to lysis in the presence of complement. The fraction of cells lysed (y) has been related to the amount of complement present (x) by the equation of von Krogh (8):

$$x = k\left(\frac{y}{1 - y}\right)^{1/n} \tag{1}$$

This relationship has been employed by several workers[3, 6, 10, 13] as a device for the evaluation of complement-activity in serum. An application of this equation to complement-fixation with type III pneumococcal carbohydrate and its homologous antiserum has been described by Mayer *et al.*[12] The equation has also been applied by the present authors in the quantitative determination of complement consumed in fixation.

THEORETICAL CONSIDERATIONS

Within the limits of 10 and 90 per cent hemolysis, Equation 1 is an adequate representation of the relationship between the fraction of cells lysed and the amount of complement initially present. If the volume of the total system is held constant, and the reaction time is sufficiently long (at least 30 minutes at 37 C.), x may be taken as the amount of complement used up in laking the cells.

If, in a given complement-fixation system, it is possible to make the reasonable assumption that the fixation of complement to the antigen-antibody complex is essentially irreversible, then the amount of complement utilized in fixation may be represented as:

$$\underset{\text{(Fixation)}}{x_F} = \underset{\text{(Blank)}}{x_B} - \underset{\text{(Test)}}{x_T} \tag{2}$$

where x_F = amount of complement fixed to the antigen-antibody complex,
x_B = amount of complement consumed in hemolysis in the absence of antigen and in the presence of the antibody, and
x_T = amount of complement available for hemolysis after fixation has occurred.

When the antigen concentration is held constant, the value of x_F may be taken as a comparative measure of the activity of specific antibody in the immune serum.

* Aided by a grant from The Commonwealth Fund, New York, N. Y.
Received for publication, September 10, 1948.

Substitution of the von Krogh equality into Equation 2 results in the following expression:

$$x_F = k_1 \left(\frac{y_B}{1 - y_B}\right)^{1/n_1} - k_2 \left(\frac{y_T}{1 - y_T}\right)^{1/n_2} \tag{3}$$

where y_B and y_T are the fractions of cells lysed by x_B and x_T respectively.

The constant k in Equation 1 depends upon the resistance to hemolysis of the sensitized erythrocytes employed. The value of the constant 1/n is believed by Hiatt[5] to be an inverse index of homogeneity of the suspension with respect to resistance. Since both k and 1/n are fixed constants for a given cell suspension, it may be seen that, in Equation 3, $k_1 = k_2$, and $1/n_1 = 1/n_2$. The equation is thus simplified to:

$$x_F = k\left[\left(\frac{y_B}{1 - y_B}\right)^{1/n} - \left(\frac{y_T}{1 - y_T}\right)^{1/n}\right] \tag{4}$$

An approximation of the value of 1/n may be obtained from the data of a serial complement-titration by use of the logarithmic form of the von Krogh equation:

$$\log x = \log k + \frac{1}{n} \log \left(\frac{y}{1 - y}\right) \tag{5}$$

For values of y within the limits of $y = 0.1$ and $y = 0.9$, the plot of log x versus log (y/1−y) is, as shown in Figure 1, approximately a straight line of slope 1/n. The value of 1/n is independent of the units of x, since any desired conversion factor is absorbed in the constant log k. It is convenient to express x in milliliters of active serum of some known dilution. If the plot is made on equal axis log-graph paper,

$$\frac{1}{n} = \tan \alpha \tag{6}$$

where α is the angle between the straight line curve and the y/1−y axis. When α is measured carefully with an accurate protractor, 1/n may be determined with two places of accuracy. In the experiments of the authors, 1/n for dilute suspensions of maximally sensitized sheep cells has been found to be within the range 0.18 to 0.32.

The constant k for the given cell suspension may also be evaluated from the logarithmic plot. The intercept of the curve with the abscissa locus $y/1-y = 1$, (or $\log (y/1-y) = 0$), has an ordinate value equal to k, since Equation 4 indicates that $\log x = \log k$ when $y/1-y = 1$. The units of k will be the same as those used for x. In fact, k is equivalent to the amount of active serum consumed in the lysis of 50 per cent of the cells, since $y/1-y = 1$ when $y = 0.5$.

A tabulation of the function $\left(\frac{y}{1 - y}\right)^{\frac{1}{n}}$ for various values of y and 1/n has been prepared by the authors and is appended to this report. When the value of 1/n has been determined for a given cell suspension, the table may be used to

obtain the values of $\left(\frac{y_B}{1-y_B}\right)^{\frac{1}{n}}$ and $\left(\frac{y_T}{1-y_T}\right)^{\frac{1}{n}}$ corresponding to observed values of y_B and y_T. These values may be substituted into Equation 4 to obtain the value of x_F. The units of x_F are relative, depending upon the value of k. Since k expresses the resistance of the cell suspension in terms of the active serum employed, it is evident that the units of x_F are determined by two factors: (a) the activity of complement in the serum, and (b) the innate resistance of the cell suspension. If either the same complement or the same cells are used for a series of fixation tests, the results, expressed in terms of x_F, will be comparable. If, on the other hand, both complement and cells are different in successive tests, the results will not be comparable.

The problem of maintaining comparable results in fixation tests performed on different days may be solved by the adoption of a laboratory standard of reference. Either the red cell suspension or the complement supply may be standardized. In either case the values of k, and hence the values of x_F, will be given an absolute significance.

The choice of standard is often a matter of convenience, depending essentially upon the number and frequency of the fixation tests performed. When tests are performed daily, it is convenient to use a preserved sheep blood as the source of red cells for the reaction. Blood obtained under aseptic conditions and preserved in an equal volume of the modified Alsever's solution described by Bukantz, Rein, and Kent[1] may be stored at 2 to 4 C. for as long as six weeks without appreciable change in the resistance of the erythrocytes. When the standard supply of red cells is replaced, it is advisable to calibrate the new supply in terms of the old, in order to maintain an accurate continuity of results.

When fixation tests are performed infrequently and at irregular intervals, the use of preserved complement allows the employment of sheep cells from a ny readily available source.

The technic for a quantitative Wassermann test used in this laboratory is given below. Standardization of the results is accomplished by the use of a commercially-available preserved (lyophile) complement. Revision of the method to employ preserved sheep blood as the standard of reference may be accomplished without difficulty. Although described in detail only for the syphilis system, the method, with appropriate modifications, may be applied to the quantitative determination of complement bound to any antigen-antibody system.

METHOD

I. Preparation of the Sheep Cell Suspension

Fresh sheep erythrocytes are washed three times in ten volumes of 0.9 per cent sodium chloride solution. The washed, packed cells are resuspended in saline to such concentration that, when 1.00 ml. of the suspension is mixed with 3.00 ml. of distilled water, the suspension becomes completely hemolyzed and the clear hemoglobin solution gives a reading of exactly 500 on the Klett-Summerson Photoelectric Colorimeter with the green (#54) filter.

This concentrated suspension is diluted with an equal volume of saline containing five units of rabbit antisheep hemolysin of a titer of at least 1:4000. The diluted suspension should then be of such concentration that, when 2.00 ml. of the suspension is mixed with 2.00 ml. of distilled water, complete hemolysis results and the clear solution again gives a reading of 500 on the colorimeter.

II. Preliminary Titration of Complement

The source of complement consists of a supply of 7 cc. ampules of Lyovac Complement.* All ampules are from the same batch, as indicated by the finishing number marked on each. One of the ampules is opened, and its contents reconstituted by the addition of 7 ml. of the accompanying buffered diluent.

TABLE 1

THE DISTRIBUTION OF COMPLEMENT AMONG TUBES FOR THE PRELIMINARY TITRATION

TUBE NO.	ML. OF 1:100 COMPLEMENT	ML. OF 0.9% SALINE
1	0.20	1.80
2	0.40	1.60
3	0.60	1.40
4	0.80	1.20
5	1.00	1.00
6	1.20	0.80
7	1.40	0.60
8	1.60	0.40

A small volume of the reconstituted serum is diluted 1:100 with 0.9 per cent saline. This diluted complement is distributed among eight clean, dry colorimeter tubes in the proportions shown in Table 1. The volume of liquid in each tube is then brought up to 2.00 ml. by the addition of the indicated amounts of saline.

To each tube, 2.00 ml. of the cell suspension is added. The tubes are shaken to provide thorough mixing of the contents, and then placed in a water bath at 37 C. for 30 minutes. At the end of this period, the tubes are centrifuged at 1500 r.p.m. for five minutes. The colorimeter reading (A) of each tube is taken. The fraction of cells hemolyzed (y) is computed as:

$$y = \frac{A}{500} \tag{7}$$

For each tube the value of the function $y/1-y$ is calculated. On $2\frac{3}{4}$ x 2 cycle equal axis log-log graph paper, the plot of x versus $y/1-y$ is constructed (see Figure 1). The value of x for each tube is taken as the amount (ml.) of 1:100 complement originally placed in the tube. The particular values of k and 1/n are obtained from the logarithmic plot as described previously. The approxi-

* Product of Sharp & Dohme, Philadelphia, Pa.

mate value of x corresponding to y = 0.85, or y/1−y = 5.67 is determined from the graph. This value (called x_{85}) then represents the amount of complement required to effect 85 per cent hemolysis of the cell suspension employed. The actual amount of reconstituted serum required is $x_{85}/100$ ml.

To prepare the complement solution for use in fixation, x_{85} ml. of the reconstituted serum is diluted with saline to a final volume of 100 ml. One ml. of this solution then contains enough complement to effect 85 per cent hemolysis. The use of the 85 per cent unit as the amount of complement employed causes all values of y to be within the effective range of the von Krogh equation. The solution should be used immediately after preparation.

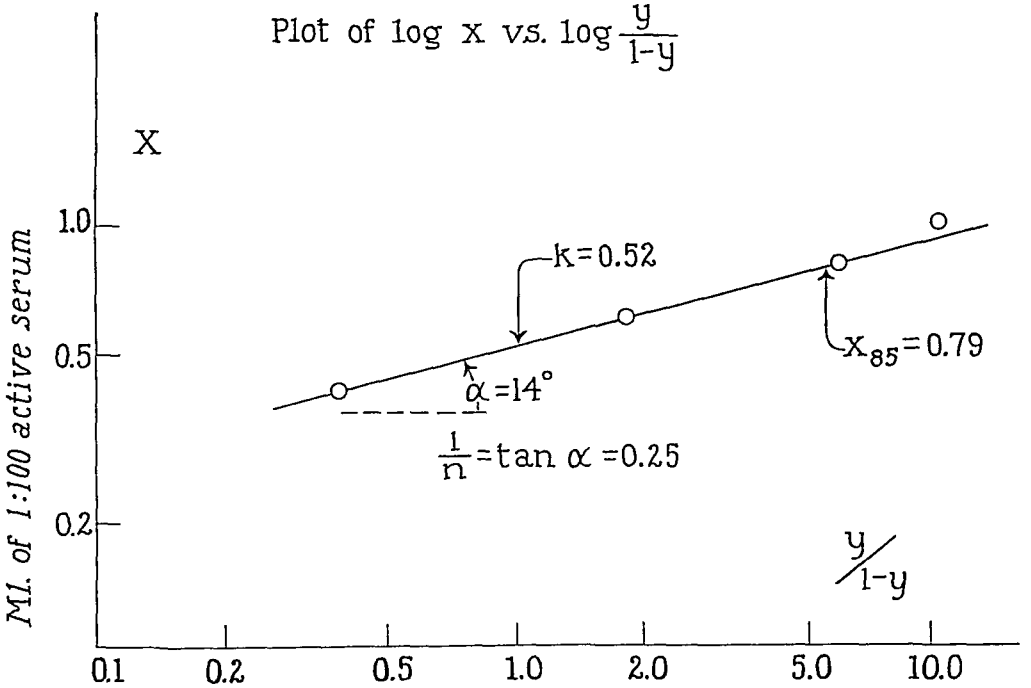


FIG. 1. Plot of the data from a typical complement-titration. The values of k, 1/n, and x_{85} are determined as indicated.

III. Preparation of the Antigen

The antigen used is the Pangborn cardiolipin, lecithin, and cholesterol (Pfansteil) in a weight ratio of 6:5:75, corresponding closely in composition with the antigen No. 42 described by Harris and Portnoy.[4] The ingredients of the antigen are mixed by adding an alcoholic solution of the cardiolipin and lecithin to a particulate suspension of cholesterol, as described by Kline.[7] The antigen is diluted 1:150 in 0.9 per cent saline just prior to use.

IV. Preparation of Serums

All serums to be tested are inactivated by heating at 56 C. for 30 minutes. The serums are tested in a dilution of 1:20.

V. Procedure

A set of six colorimeter tubes is assembled for each serum to be tested. Three of these tubes are designated as "blanks", the other three as "tests". The reagents are added to the tubes in the order indicated in Table 2. After thorough mixing, the tubes are placed in the water bath at 37 C. for one hour. At the end of this period, the tubes are removed from the bath, and 2.00 ml. of the cell suspension is added to each tube. After thorough mixing, the tubes are replaced in the bath for a secondary incubation period of 30 minutes. After this period, the tubes are centrifuged at 1500 r.p.m. for five minutes, and the colorimeter reading of the clear supernate in each tube is taken.

For each set of blanks and tests, the values of y_B and y_T are computed as indicated in Equation 7. Using the values of k and 1/n obtained in the preliminary complement titration, and referring to the proper section of Table 5, the value of x_F for each test serum is calculated from Equation 4.

TABLE 2

OUTLINE OF PROCEDURE IN THE FIXATION TEST

BLANK	TEST
1.00 ml. diluted complement 0.50 ml. 1:20 test serum 0.50 ml. saline	1.00 ml. diluted complement 0.50 ml. 1:20 test serum 0.50 ml. 1:150 antigen complement
Incubation, one hour at 37 C.	
2.00 ml. cell suspension	2.00 ml. cell suspension

Incubation, 30 minutes at 37 C.
Centrifugation at 1500 r.p.m. for five minutes
Colorimeter reading recorded

A summary of the results obtained in the complement-fixation test with two syphilitic serums, using three different cell suspensions on the same day, is shown in Table 3. The comparison of results before and after introduction of the constant k indicates the extent of improvement effected by attention paid to the value of this constant. The variability evidenced among the three cell suspensions is representative of that encountered among suspensions of cells prepared on different days and from different sheep.

To determine the extent of variability among different ampules of the standard lyophile complement, twelve ampules were chosen at random, and their contents were reconstituted and titrated with a single cell suspension. The results of the titration are shown in Table 4. The variability among tubes is seen to average about ±3 per cent. Volumetric errors in apportioning the serum into the tubes prior to drying are probably responsible for most of the variability observed.

The stability of lyophile complement over extended periods of time is the most important factor in its effectiveness as a standard. According to Eagle,

Strauss and Steiner,[2] fresh serum which has been dried rapidly from the frozen state and stored *in vacuo* at 2 to 5 C. will retain its complement-activity for at

TABLE 3

COMPARISON OF REAGIN TITERS BEFORE AND AFTER APPLICATION OF THE CORRECTION CONSTANT K

CELL SUSPENSION	SERUM A		SERUM B	
	x_F/k	x_F	x_F/k	x_F
I. k 0.66 1/n 0.23	0.80	0.53	0.77	0.51
II. k 0.55 1/n 0.25	1.08	0.59	1.03	0.57
III. k 0.45 1/n 0.32	1.30	0.59	0.92	0.54
Mean	1.06	0.57	0.91	0.54
Average deviation from mean (%)	16.3	4.6	9.9	3.7

Average deviation before applying k 13.1%
Average deviation after applying k 4.2%

TABLE 4

VARIABILITY OF SHARP AND DOHME "LYOVAC COMPLEMENT" AMONG AMPULES FROM THE SAME BATCH

AMPULE NO.	k	DEVIATION FROM AVERAGE
1	0.48	+0.006
2	0.48	+0.006
3	0.46	−0.014
4	0.46	−0.014
5	0.47	−0.004
6	0.46	−0.014
7	0.47	−0.004
8	0.48	+0.006
9	0.48	+0.006
10	0.51	+0.036
11	0.45	−0.024
12	0.49	+0.016
Average	0.474	±0.0125

Mean deviation = ± 2.6%

Standard deviation = $\pm\sqrt{\frac{\Sigma\Delta X^{-2}}{n-1}}$ = ±0.0162 or ±3.4 %

least two years without significant change. The preparation of a stable lyophile complement is a process which requires equipment not found in the ordinary serologic laboratory. For this reason the commercial preparation has been se-

TABLE 5

TABULATION OF THE FUNCTION $\left(\frac{y}{1-y}\right)^{1/n}$ FOR VARIOUS VALUES OF y AND 1/n

(The following tables have been prepared by graphical interpolation. The values are given in two decimals to be consistent with experimental accuracy.)

y	1/n															
	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33
0.10	0.66	0.64	0.63	0.62	0.61	0.60	0.59	0.58	0.57	0.55	0.54	0.53	0.52	0.51	0.50	0.49
0.11	0.67	0.65	0.64	0.63	0.62	0.61	0.60	0.59	0.58	0.56	0.55	0.54	0.53	0.52	0.51	0.50
0.12	0.68	0.66	0.66	0.64	0.63	0.62	0.61	0.60	0.59	0.57	0.57	0.56	0.55	0.54	0.53	0.51
0.13	0.70	0.69	0.67	0.66	0.65	0.64	0.63	0.62	0.60	0.59	0.58	0.57	0.56	0.55	0.54	0.53
0.14	0.71	0.70	0.69	0.67	0.66	0.65	0.64	0.63	0.62	0.60	0.60	0.59	0.58	0.57	0.56	0.55
0.15	0.72	0.71	0.70	0.69	0.68	0.67	0.66	0.65	0.63	0.62	0.61	0.60	0.59	0.58	0.57	0.56
0.16	0.73	0.72	0.71	0.70	0.69	0.68	0.67	0.66	0.64	0.63	0.62	0.61	0.60	0.59	0.58	0.57
0.17	0.74	0.73	0.72	0.71	0.70	0.69	0.68	0.67	0.65	0.64	0.64	0.63	0.62	0.61	0.60	0.59
0.18	0.75	0.74	0.73	0.72	0.71	0.70	0.69	0.68	0.66	0.65	0.65	0.64	0.63	0.62	0.61	0.60
0.19	0.76	0.75	0.74	0.73	0.72	0.71	0.70	0.70	0.68	0.67	0.66	0.65	0.65	0.64	0.62	0.61
0.20	0.77	0.76	0.75	0.74	0.73	0.72	0.71	0.71	0.70	0.68	0.67	0.66	0.66	0.65	0.64	0.62
0.21	0.78	0.77	0.75	0.75	0.74	0.73	0.72	0.72	0.71	0.69	0.69	0.68	0.67	0.66	0.65	0.64
0.22	0.79	0.78	0.76	0.75	0.75	0.74	0.73	0.73	0.72	0.70	0.70	0.69	0.68	0.67	0.66	0.65
0.23	0.80	0.79	0.78	0.77	0.76	0.75	0.74	0.74	0.73	0.71	0.71	0.70	0.69	0.68	0.67	0.66
0.24	0.81	0.80	0.79	0.78	0.77	0.76	0.75	0.75	0.74	0.72	0.72	0.71	0.71	0.70	0.69	0.67
0.25	0.81	0.80	0.80	0.79	0.78	0.77	0.76	0.76	0.75	0.74	0.73	0.72	0.72	0.71	0.70	0.69
0.26	0.82	0.81	0.81	0.80	0.79	0.78	0.77	0.77	0.76	0.75	0.74	0.73	0.73	0.72	0.71	0.70
0.27	0.83	0.82	0.81	0.81	0.80	0.79	0.78	0.78	0.77	0.76	0.75	0.74	0.74	0.73	0.72	0.71
0.28	0.84	0.83	0.82	0.81	0.80	0.80	0.79	0.79	0.78	0.77	0.77	0.76	0.75	0.74	0.73	0.72
0.29	0.85	0.83	0.83	0.82	0.82	0.81	0.81	0.80	0.79	0.78	0.78	0.77	0.76	0.75	0.74	0.73
0.30	0.85	0.84	0.84	0.83	0.83	0.82	0.82	0.81	0.80	0.79	0.79	0.78	0.78	0.77	0.76	0.74
0.31	0.86	0.85	0.84	0.84	0.83	0.83	0.82	0.82	0.81	0.80	0.80	0.79	0.79	0.78	0.77	0.76
0.32	0.87	0.86	0.85	0.85	0.84	0.84	0.83	0.83	0.82	0.81	0.81	0.80	0.80	0.79	0.78	0.77
0.33	0.88	0.87	0.86	0.86	0.85	0.85	0.84	0.84	0.83	0.82	0.82	0.81	0.81	0.80	0.79	0.78
0.34	0.88	0.87	0.87	0.87	0.86	0.86	0.85	0.85	0.84	0.83	0.83	0.82	0.82	0.81	0.80	0.79
0.35	0.89	0.88	0.88	0.88	0.87	0.87	0.86	0.86	0.85	0.84	0.84	0.83	0.83	0.82	0.81	0.80
0.36	0.90	0.89	0.88	0.88	0.87	0.87	0.86	0.86	0.85	0.85	0.85	0.84	0.84	0.83	0.82	0.81
0.37	0.91	0.90	0.89	0.89	0.88	0.88	0.87	0.87	0.86	0.86	0.86	0.85	0.85	0.84	0.83	0.82
0.38	0.92	0.91	0.90	0.90	0.89	0.89	0.88	0.88	0.87	0.87	0.87	0.86	0.86	0.85	0.84	0.83
0.39	0.93	0.92	0.91	0.91	0.90	0.90	0.89	0.89	0.88	0.88	0.88	0.87	0.87	0.86	0.85	0.84
0.40	0.93	0.92	0.92	0.92	0.91	0.91	0.90	0.90	0.89	0.89	0.89	0.88	0.88	0.87	0.87	0.86
0.41	0.94	0.93	0.93	0.93	0.92	0.92	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.89	0.89	0.88
0.42	0.95	0.94	0.94	0.94	0.93	0.93	0.92	0.92	0.92	0.92	0.91	0.91	0.91	0.90	0.89	0.89
0.43	0.96	0.95	0.95	0.95	0.94	0.94	0.93	0.93	0.93	0.93	0.92	0.92	0.92	0.91	0.90	0.90
0.44	0.96	0.96	0.95	0.95	0.95	0.95	0.94	0.94	0.94	0.94	0.93	0.93	0.93	0.92	0.91	0.91

0.45	0.97	0.97	0.96	0.96	0.96	0.96	0.95	0.95	0.95	0.95	0.94	0.94	0.94	0.93	0.93	0.92
0.46	0.97	0.97	0.97	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.95	0.95	0.94	0.94	0.93
0.47	0.98	0.98	0.98	0.98	0.98	0.97	0.97	0.97	0.97	0.97	0.97	0.96	0.96	0.96	0.96	0.95
0.48	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.97	0.96	0.97	0.97
0.49	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.98	0.98
0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.51	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.02	1.02	1.02	1.02	1.02	1.02	1.03
0.52	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.03	1.03	1.03	1.03	1.03	1.04	1.04
0.53	1.02	1.02	1.02	1.02	1.03	1.03	1.03	1.03	1.03	1.04	1.04	1.04	1.04	1.05	1.05	1.05
0.54	1.03	1.03	1.03	1.03	1.04	1.04	1.04	1.04	1.04	1.05	1.05	1.05	1.05	1.06	1.06	1.07
0.55	1.03	1.03	1.04	1.04	1.04	1.05	1.05	1.05	1.05	1.06	1.06	1.06	1.06	1.07	1.07	1.08
0.56	1.04	1.04	1.05	1.05	1.05	1.05	1.06	1.06	1.06	1.07	1.07	1.07	1.08	1.09	1.09	1.10
0.57	1.05	1.05	1.06	1.06	1.06	1.07	1.07	1.07	1.08	1.08	1.08	1.08	1.09	1.10	1.11	1.12
0.58	1.06	1.06	1.07	1.07	1.07	1.08	1.08	1.08	1.09	1.09	1.10	1.10	1.11	1.12	1.13	1.13
0.59	1.07	1.07	1.08	1.08	1.08	1.09	1.09	1.09	1.10	1.11	1.11	1.11	1.12	1.13	1.14	1.15
0.60	1.07	1.08	1.09	1.09	1.10	1.10	1.10	1.11	1.11	1.12	1.12	1.12	1.13	1.14	1.15	1.17
0.61	1.08	1.09	1.10	1.10	1.11	1.11	1.12	1.12	1.12	1.13	1.13	1.14	1.15	1.16	1.17	1.18
0.62	1.09	1.10	1.11	1.11	1.12	1.12	1.13	1.13	1.14	1.14	1.15	1.15	1.16	1.17	1.18	1.19
0.63	1.10	1.11	1.12	1.12	1.13	1.13	1.14	1.14	1.15	1.16	1.16	1.16	1.17	1.18	1.19	1.21
0.64	1.11	1.12	1.13	1.13	1.13	1.14	1.14	1.15	1.16	1.17	1.18	1.18	1.19	1.20	1.21	1.23
0.65	1.12	1.13	1.13	1.14	1.14	1.15	1.15	1.16	1.17	1.18	1.19	1.20	1.21	1.22	1.23	1.24
0.66	1.13	1.14	1.14	1.15	1.15	1.16	1.16	1.17	1.18	1.20	1.21	1.22	1.22	1.23	1.24	1.26
0.67	1.14	1.15	1.15	1.16	1.16	1.17	1.18	1.19	1.20	1.21	1.22	1.23	1.24	1.25	1.26	1.27
0.68	1.15	1.16	1.17	1.18	1.18	1.19	1.20	1.21	1.22	1.23	1.23	1.25	1.26	1.26	1.27	1.28
0.69	1.16	1.17	1.18	1.19	1.19	1.20	1.21	1.22	1.23	1.24	1.25	1.26	1.27	1.28	1.29	1.30
0.70	1.17	1.18	1.19	1.20	1.21	1.22	1.23	1.24	1.25	1.26	1.27	1.28	1.29	1.30	1.31	1.32
0.71	1.18	1.19	1.20	1.21	1.22	1.23	1.24	1.25	1.26	1.28	1.29	1.30	1.31	1.32	1.33	1.34
0.72	1.19	1.20	1.21	1.22	1.23	1.24	1.25	1.26	1.28	1.30	1.31	1.32	1.33	1.34	1.35	1.36
0.73	1.20	1.21	1.23	1.23	1.24	1.25	1.26	1.27	1.29	1.32	1.33	1.34	1.35	1.36	1.37	1.38
0.74	1.21	1.22	1.24	1.25	1.26	1.27	1.28	1.29	1.31	1.33	1.35	1.36	1.37	1.38	1.39	1.41
0.75	1.22	1.23	1.25	1.27	1.28	1.29	1.30	1.31	1.33	1.35	1.37	1.39	1.40	1.41	1.42	1.43
0.76	1.23	1.24	1.26	1.29	1.30	1.31	1.32	1.33	1.35	1.37	1.38	1.40	1.42	1.43	1.44	1.46
0.77	1.25	1.26	1.28	1.30	1.32	1.33	1.34	1.35	1.37	1.39	1.41	1.42	1.44	1.46	1.47	1.49
0.78	1.27	1.28	1.31	1.32	1.34	1.35	1.36	1.37	1.39	1.42	1.43	1.45	1.47	1.49	1.50	1.52
0.79	1.28	1.30	1.33	1.33	1.36	1.37	1.38	1.39	1.41	1.44	1.46	1.48	1.50	1.52	1.53	1.54
0.80	1.29	1.32	1.34	1.35	1.38	1.39	1.40	1.41	1.43	1.46	1.48	1.51	1.53	1.55	1.56	1.58
0.81	1.32	1.34	1.36	1.37	1.39	1.41	1.42	1.43	1.46	1.49	1.51	1.53	1.55	1.57	1.59	1.61
0.82	1.33	1.35	1.38	1.40	1.41	1.43	1.44	1.45	1.49	1.52	1.54	1.56	1.58	1.60	1.62	1.64
0.83	1.34	1.37	1.40	1.42	1.44	1.46	1.47	1.48	1.51	1.55	1.57	1.60	1.62	1.64	1.66	1.68
0.84	1.37	1.39	1.42	1.45	1.47	1.49	1.50	1.51	1.54	1.58	1.61	1.63	1.65	1.68	1.70	1.73
0.85	1.38	1.41	1.45	1.48	1.50	1.52	1.53	1.54	1.57	1.61	1.64	1.67	1.69	1.72	1.74	1.77
0.86	1.40	1.44	1.47	1.51	1.53	1.55	1.56	1.58	1.60	1.65	1.68	1.71	1.74	1.77	1.79	1.82
0.87	1.42	1.46	1.49	1.54	1.56	1.58	1.59	1.61	1.63	1.68	1.72	1.75	1.78	1.81	1.83	1.87
0.88	1.44	1.49	1.52	1.56	1.59	1.61	1.63	1.65	1.68	1.73	1.77	1.80	1.83	1.86	1.89	1.93
0.89	1.47	1.51	1.54	1.59	1.62	1.64	1.66	1.68	1.73	1.78	1.81	1.85	1.88	1.93	1.96	1.98
0.90	1.50	1.55	1.59	1.63	1.66	1.68	1.70	1.72	1.77	1.83	1.87	1.91	1.95	1.99	2.02	2.06

lected for use by the authors. In addition to serving as a standard of reference, the lyophilized product constitutes the total supply of complement required for all fixation tests, thus relieving the laboratory of the necessity of maintaining guinea pigs.

In the adaptation of the quantitative fixation method to other complement-fixing systems, it may be desirable to provide a larger excess of complement in the primary reaction. Small aliquot samples, each containing less than one 100 per cent unit of unconsumed complement, may then be removed from the system and tested in the secondary reaction with sensitized red cells. This technic, incorporated in the method described by Mayer *et al.*,[12] is of particular advantage where the antibody activity of the antiserums tested may vary over an extremely wide range.

The inclusion of an excess of Mg^{++} and Ca^{++} ions in the diluent, as proposed by Mayer *et al.*,[11] is known to result in an increased complement-activity. The well known reactivity of bivalent metallic ions with serum phosphatides, as noted by Maltaner and de Almeida,[9] would suggest that the addition of abnormal amounts of these ions should be attended with caution, particularly when the fixation reaction involves lipid antigens.

SUMMARY

The von Krogh equation,

$$x = \left(\frac{y}{1 - y}\right)^{\frac{1}{n}}$$

which relates the concentration of complement (x) and the degree of hemolysis (y), is applied to the evaluation of the hemolytic equivalent of complement in fixation. The amount of complement fixed to an antigen-antibody complex is given as:

$$\underset{\text{(Fixation)}}{x_F} = \underset{\text{(Blank)}}{x_B} - \underset{\text{(Test)}}{x_T}$$

where x_F is the amount of complement fixed, and x_B and x_T are the amounts of complement consumed in hemolysis in the absence and in the presence of antigen.

If the antigen concentration is kept constant, then the quantity x_F may be taken as a measure of the activity of the antibody. The value of x_F may be determined by the von Krogh equation after the constants k and 1/n of this equation have been evaluated.

A tabulation of the function $\left(\frac{y}{1 - y}\right)^{\frac{1}{n}}$ for various values of y and $1/n$ is appended to assist in the calculations.

The method is exemplified in the quantitative Wassermann test described. Comparability of results can be maintained (1) by the use of a standard complement or (2) by aseptically drawn and preserved sheep red cells.

The introduction of a standard is offered as a procedure to avoid the errors

incurred by the variability of red cell suspensions. The latter can also be improved by the use of a preservative.

The method, with appropriate modifications, may be applied to any complement-fixation system.

REFERENCES

1. BUKANTZ, S. C., REIN, C. R., AND KENT, J. F.: Studies in complement fixation; preservation of sheep's blood in citrate-dextrose mixtures (modified Alsever's solution) for use in complement fixation reaction. J. Lab. and Clin. Med., **31:** 394–399, 1946.
2. EAGLE, H., STRAUSS, H., AND STEINER, R.: Use in Wassermann reaction of uniform and stable dehydrated complement. Am. J. Clin. Path., **5:** 173–179, 1935.
3. ECKER, E. E., HIATT, C. W., AND BARR, L. M.: Effect of pregnancy on complement of guinea-pigs. J. Lab. and Clin. Med., **32:** 287–291, 1947.
4. HARRIS, A., AND PORTNOY, J.: Cardiolipin antigens in the Kolmer complement fixation test for syphilis. Ven. Dis. Inform., **25:** 353–361, 1944.
5. HIATT, C. W.: Certain mathematical aspects of the susceptibility of erythrocytes to lysis. Proc. Soc. Exper. Biol. and Med., **66:** 279–281, 1947.
6. KENT, J. F., BUKANTZ, S. C., AND REIN, C. R.: Studies in complement fixation; spectrophotometric titration of complement; construction of graphs for direct determination of the 50 per cent hemolytic unit. J. Immunol., **53:** 37–50, 1946.
7. KLINE, B. S.: Cardiolipin antigen in microscopic slide precipitation test for syphilis. Am. J. Clin. Path., **16:** 68–80, 1946.
8. VON KROGH, M.: Colloidal chemistry and immunology. J. Infect. Dis., **19:** 452–477, 1916.
9. MALTANER, F., AND DE ALMEIDA, J. O.: The effect of Mg++ on the complementary coagulative properties of blood serum. Fed. Proc., **7:** 307–308, 1948.
10. MAYER, M. M., EATON, B. B., AND HEIDELBERGER, M.: Spectrophotometric standardization of complement for fixation tests. J. Immunol., **53:** 31–35, 1946.
11. MAYER, M. M., OSLER, A. G., BIER, O. G., AND HEIDELBERGER, M.: Activating effect of magnesium and other cations on hemolytic function of complement. J. Exper. Med., **84:** 535–548, 1946.
12. MAYER, M. M., OSLER, A. G., BIER, O. G., AND HEIDELBERGER, M.: Quantitative studies of complement fixation. Proc. Soc. Exper. Biol. and Med., **65:** 66–68, 1947.
13. WADSWORTH, A., MALTANER, E., AND MALTANER, F.: Quantitative determination of fixation of complement by immune serum and antigen. J. Immunol., **21:** 313–340, 1931.

POOLED SHEEP CELLS FOR USE IN THE HEMOLYTIC SYSTEM OF THE COMPLEMENT-FIXATION TEST*

THELMA F. MURASCHI, B.S., AND VICTOR N. TOMPKINS, M.D.

From Division of Laboratories and Research, New York State Department of Health, Albany, New York

In the performance of complement-fixation tests, it is essential to have components of the hemolytic system relatively constant in activity from day to day. In order to provide reagents of such uniformity, guinea pig serums which are to serve as a source of complement, and immune rabbit serums which are to serve as a source of antisheep amboceptor, can each be pooled in large quantity and preserved for use over prolonged periods. A similar supply of the third reagent, sheep erythrocytes, can generally be made available by collecting a large quantity of blood from one sheep in an appropriate preserving solution. However, in large laboratories where the amount of blood required for each day's work often approaches the maximum which can safely be withdrawn from a single sheep, the volume necessary for extended use can be made available only if preserved blood from several sheep is pooled.

Until early in 1947, it was the practice at this laboratory to use approximately 350 ml. of blood collected from a single sheep for each day's work. Complement unit values varied from day to day as a result of differences in the rate of hemolysis of the cells from each sheep. For example, with the cells from seven sheep tested on one day, the hemolytic units (ml. of complement required for 50 per cent hemolysis) were 0.00143, .00207, .00181, .00149, .00145, .00197, .00156, and with one lot of pooled cells 0.00155.

Following the work of Bukantz, Rein, and Kent,[1] it was found that sheep erythrocytes collected in modified Alsever's solution were satisfactorily preserved for at least three weeks. Study was then directed to the use of pooled preserved blood, dispensed aseptically in amounts sufficient for a single day's work.

SELECTION OF ANIMALS

It was customary, when small numbers of guinea pig serums were to be pooled for use as the source of complement, to pretest each serum with *unsensitized* sheep cells for evidence of natural antisheep amboceptor. Hemolysis in the test indicated the presence of a lysin, introduced presumably by the guinea pig serum, and necessitated omission of the serum from the complement pool. Differences between pools of sheep blood were first noted in the routine performance of this test. With individual sheep bleedings, only rarely had a guinea pig serum been found to induce hemolysis of unsensitized cells. In the case of pooled blood, however, an occasional combination of sheep bleedings was encountered with which all guinea pig serums appeared unsatisfactory. It was thus apparent that the serum of each sheep would need to be tested against the cells of every other

* Received for publication, September 20, 1948.

sheep in a pool. The test, essentially one of compatibility, was performed by mixing 0.1 ml. of fresh sheep serum with 0.1 ml. of approximately 5 per cent suspension of erythrocytes from different sheep, adding 0.1 ml. of guinea pig serum, and allowing 15 minutes at 37 C. for hemolysis.

Fresh bleedings for serum were found to be necessary, since lytic activity declined during storage, even though the serum was refrigerated, and was destroyed when serum was heated at 56 C. for 30 minutes. Somewhat different behavior was noted when the pooled, whole blood was stored. Hemolysis observed in tests of cells washed soon after pooling was consistent and often unaltered in intensity when samples were tested at intervals during several weeks' storage. Contact between susceptible erythrocytes and lytic serum may be responsible for the more enduring activity.

In our experience, the property responsible for lysis is not demonstrable as an agglutinin; whether it be of the iso-antibody or acquired antibody type has not been determined. Sheep blood has been found to vary in the extent and degree of hemolytic activity, though the presence of hemolysin is not transitory but consistent. In tests of serum from 134 sheep, evidence of a lysin for cells of some other sheep was detected in seventeen, or 12 per cent. Each serum was tested for lytic activity with cells from only a limited number of sheep, not with cells from each of the other 133 sheep. Satisfactory pools of blood are now prepared on the basis of results of the test for compatibility.

The pretesting and selection of sheep to furnish blood for pools can be omitted if it is practicable to store individual bleedings and combine cells only after washing to remove serum constituents.

COMPLEMENT TITRATION

Other things being equal, differences in milliliters of complement required for hemolysis of a given volume of maximally sensitized sheep erythrocytes should, with rare exception, reflect red cell differences. Complement units, therefore, were used as the indexes of variability in the comparison of pools of blood with blood from a single sheep. The standard red cell suspension employed in all tests was prepared by suspending the washed erythrocytes in physiologic saline in a 5 per cent concentration. The accuracy of the dilution was checked by a hemoglobin determination in a photoelectric colorimeter.

Technic

The technic of the complement titration was a modification of that described in Standard Methods.[2] Two samples of a 1:40 dilution of pooled guinea pig serum were titrated in amounts of 0.09, 0.08, 0.07, 0.06, 0.05, and 0.04 ml. Physiologic saline was added to raise the volume to 0.3 ml., and 0.2 ml. of a maximally sensitized 5 per cent sheep red cell suspension was added. Incubation was in a water bath at 37 C. Hemolysis occurring in each tube was recorded at the end of 15 minutes. Limiting values for the milliliters of guinea pig serum necessary to induce 50 per cent hemolysis of 0.2 ml. of a maximally sensitized 5 per cent suspension of sheep erythrocytes have been set at 0.001 ml. and 0.002

in tests allowing 15 minutes for hemolysis. The most satisfactory range is that from about 0.0013 ml. to 0.0017 ml.

RESULTS

Daily hemolytic units of guinea pig serum obtained with pooled sheep cells have proved much less variable than those obtained with cells from single sheep.

TABLE 1

INFLUENCE OF POOLING ON COMPLEMENT REQUIREMENTS FOR 50 PER CENT HEMOLYSIS

		MILLILITERS OF GUINEA PIG SERUM REQUIRED FOR 50 PER CENT HEMOLYSIS				
Cells from single sheep	Pool of cells from several sheep	Combined cells of single sheep and pool				Remarks
		ratio 1:1	ratio 1:2	ratio 1:3	ratio 1:4	
0.00198	0.00159	.00186 (.00178)*	.00175 (.00172)	.00168 (.00169)	.00168 (.00167)	Satisfactory in dilution
0.00215	0.00159	.00196 (.00187)	.00182 (.00178)	.00173 (.00175)	.00175 (.00170)	Satisfactory in dilution
0.00250	0.00159	.00201 (.00204)	.00179 (.00189)	.00183 (.00182)	.00159 (.00177)	Satisfactory in dilution
0.00228	0.00159	.00196 (.00193)	.00196 (.00182)	.00181 (.00176)	.00182 (.00175)	May be satisfactory on further dilution

* Figures in parentheses represent theoretical values derived mathematically.

It has been our experience that the titration procedure is subject to a possible 10 per cent error, so that differences in the fourth and fifth digit beyond the decimal point may be without significance.

	COMPLEMENT TITRATION					
1:40 dilution tested (ml.)	0.09	0.08	0.07	0.06	0.05	0.04
Undiluted serum represented (ml.)	0.00225	0.002	0.00175	0.0015	0.00125	0.001
Percentage hemolysis after incubation	100	95	85	70	40	20

Moreover, unit amounts of complement for pooled cells generally approach the ideal, 0.0015 ml., and only occasionally exceed the desirable range, 0.0013 to 0.0017 ml. Pooled sheep blood, shipped regularly without refrigeration to a branch laboratory 150 miles distant, has also been satisfactory in routine tests.

The implications are that, under optimum conditions of testing, different lots of guinea pig serum are similar in hemolytic activity. Evidently, variability is actually a function of sheep cells. In the past, and with the use of a single sheep bleeding for each day's work, failure to obtain adequate hemolysis of sheep cells, a too common occurrence, was attributed to complement deficiency of the serum. This conclusion appeared justified in view of the fact that sheep employed rou-

tinely were selected only after preliminary tests (for complement requirements) had indicated that the cells were suitable. Adjustments necessary when an unsatisfactory unit of complement was obtained resulted in a considerable delay in the performance of the day's test,—a rare occurrence now.

Fewer sheep are now rejected because of the amount of guinea pig serum required to produce 50 per cent hemolysis. Cells which of themselves are undesirable for this reason, when added to a satisfactory pool of cells in a ratio to allow adequate dilution, appear to have little or no harmful effect. The dilution necessary in such instances may be determined mathematically, given complement requirements of the cells from the single sheep and the cells of the pool to which they are to be added. In the same manner, the milliliters of guinea pig serum essential to the final pool for 50 per cent hemolysis may usually be predicted (Table 1).

Pooled, preserved cells to which Merthiolate was added in concentrations 1:2,000 and 1:10,000 as used for maintenance of sterility in biologic products were tested over a period of two weeks. No evidence of increased tendency to hemolysis was observed on washing or in titration with complement and amboceptor. Merthiolate may prove helpful as an antibacterial agent for cells collected in a citrate-glucose solution.

SUMMARY

Pooled, preserved sheep blood pretested for compatibility has been used as a source of erythrocytes in the quantitative complement-fixation test. A uniform activity not obtained with blood from a single sheep has resulted, and the standardization of the hemolytic system has been greatly simplified.

REFERENCES

1. Bukantz, S. C., Rein, C. R., and Kent, J. F.: Studies in complement fixation; preservation of sheep's blood in citrate dextrose mixtures (modified Alsever's solution) for use in the complement fixation reaction. J. Lab. and Clin. Med., **31**, 394–399, 1946.
2. Wadsworth, A. B.: Standard Methods of the Division of Laboratories and Research of the New York State Department of Health. Ed. 3. Baltimore: The Williams and Wilkins Company, pp. 383–386, 1947.

SPECIFICITY OF CARDIOLIPIN ANTIGENS IN TESTS FOR SYPHILIS*

SARA C. McDEARMAN, A.B., AND JAMES E. COTTRELL, M.D.

From the Laboratory Service and the Medical Service, Veterans Administration Medical Teaching Group, Kennedy Hospital, Memphis, Tennessee

The use of cardiolipin antigens as a standard procedure in the serodiagnosis of syphilis by the Army Medical Department,[9] and the Venereal Disease Research Laboratory, U.S.P.H.S.[3, 4] is an indication of the superiority, in some respects at least, of this phospholipid over the ordinary tissue-extract antigens. Standard flocculation and complement-fixation technics with tissue-extract antigens were used as patterns for adapting cardiolipin antigens. Numerous workers have reported the constant reproducibility of the essentially chemically pure cardiolipin and the stability of the antigens; their reports suggest that the sensitivity, and possibly the specificity, of these antigens in tests for syphilitic reagin are greater than those of standard antigens. Serologists are generally agreed on the increased sensitivity obtained. Most observers, however, have been rightfully cautious concerning the specificity, stating that it is as good as, and usually superior to, that obtained with the tissue-extract antigens. Stout[14] found false positive reactions with the cardiolipin antigens in a small series of patients, indicating that such nonspecific reactions do occur in the absence of syphilis. Others have reported an increase in the number of weakly positive or doubtful reactions. Many of these studies have been conducted in laboratories receiving serums from various sources from large groups of persons whose clinical and social histories were unavailable. In some reports there have been correlations of serologic and clinical findings. The incidence of nonspecific reactions in malaria has been well studied by Rein and Kent,[12] and Kent, Culwell, Coatney, and Cooper.[6] They have noted the dramatic increase in specificity with cardiolipin antigens, especially with a complement-fixation technic, using large series of serums from patients with malaria.

This study was thought advisable since a large number of patients representing all types of illness would furnish clinically diverse testing material.

METHOD

The serums tested in this study represent an unselected group of persons, including patients admitted to the hospital for treatment, veterans referred for rating purposes, and hospital staff workers with no detectable evidence of disease.

A battery of serologic tests has been used routinely, including Standard Kahn, Kolmer complement-fixation, cardiolipin microflocculation (V.D.R.L.),[3] and

* Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

Read by title at the Twenty-Seventh Annual Meeting of the American Society of Clinical Pathologists, in Chicago, October 14, 1948.

Received for publication, September 29, 1948.

cardiolipin complement-fixation.[5] Only those serums have been included in this study which have been tested by all four technics and a few serums have been subjected to an additional cardiolipin microflocculation technic.[11] The results of the tests were read by the same three workers and have been recorded as weakly positive (plus-minus, 1 plus) and positive (2 plus, 3 plus, 4 plus). Quantitative tests, using two-fold serum dilutions, were performed on all serums giving a reactivity of 4 plus in any one of the tests. Suitable positive, weakly positive, and negative control serums and antigen controls were subjected to the battery of tests each day.

Precautions were taken to assure that the sensitivity levels of the antigens* used did not vary, so as not to alter the final results of the study. Antigens were prepared in 50 ml. amounts and each batch was compared with the original one for sensitivity on known syphilitic serums. Fresh antigen emulsions were prepared each day and were used within eight hours. Technics as proposed by the author serologists were strictly adhered to at all times.

Between June 16, 1947 and August 1, 1948, 60,226 tests were performed on 15,019 serums. Those serums giving a reactivity of plus-minus or greater in one or more cardiolipin tests and no reactivity with Kahn and Kolmer tests have been included for evaluation in this study. Wherever possible the clinical and social histories of patients giving this type of serum reactivity were reviewed for any objective evidence of syphilitic infection or history of such infection. A subject was designated "syphilitic" if he had one or more of the following findings: positive darkfield for *Treponema pallidum*; primary lesion; clinical and/or spinal fluid evidence of neurosyphilis (Kolmer complement-fixation, colloidal gold curve); clinical signs and symptoms as suggested by Stokes, Beerman, and Ingraham[13] for diagnosis of cardiovascular syphilis; a history of previous syphilitic infection which was considered, from the clinical aspect, to be sufficiently definite and reliable to be acceptable. For the purpose of this study, "presumed nonsyphilitics" have been included in the "negative" group.

RESULTS AND DISCUSSION

Of the 15,019 serums tested, 991 gave plus-minus or stronger reactions with at least one cardiolipin technic in the absence of any reaction with the Kahn and Kolmer antigens. These 991 serums represent 654 persons who have been classified into five groups as indicated in Table 1.

Approximately 3300 of the 15,019 serums were from presumably normal persons who came to this laboratory for routine health ratings, as prospective employees or blood donors. Their venereal disease histories were not taken. The 119 cardiolipin reactors among these 3300 persons included kitchen helpers and ward attendants (mainly Negro); no reactions were found among a large group of doctors, nurses, and other professional workers.

Clinical and social histories of 81 hospital patients were inadequate or un-

* The cardiolipin and lecithin solutions were obtained from Lederle Laboratories Division, American Cyanamid Company, New York, N. Y.

available and no attempt was made to correlate the clinical and serologic findings in these cases.

Of the 654 cardiolipin reactors, 454 hospital patients were studied to determine the existence of a syphilitic lesion or evidence of previous syphilitic infection.

TABLE 1

ANALYSIS OF 654 PATIENTS WITH NEGATIVE KAHN AND KOLMER TESTS AND POSITIVE OR WEAKLY POSITIVE CARDIOLIPIN REACTIONS

	NUMBER OF PATIENTS	WEAKLY POSITIVE REACTORS (±, 1+)	POSITIVE REACTORS (2+, 3+, 4+)
Syphilitic group	196	45	151
"Negative" group	258	125	133
Undetermined group	200	88	112
Total	654	258	396

TABLE 2

ANALYSIS OF 454 PATIENTS WHOSE CLINICAL AND SOCIAL HISTORIES WERE REVIEWED

	NUMBER OF PATIENTS	RACE		WEAKLY POSITIVE REACTORS (±, 1+)	POSITIVE REACTORS (2+, 3+, 4+)	MORE THAN ONE SPECIMEN OBTAINED	
		W	N			No. of Patients	Patients with Positive Kahn or Kolmer
Syphilitic group	196 (43.2%)	64	132	45	151	123	51
Primary lesion	7	3	4		7	6	4
Secondary rash	1	1			1	1	1
Tertiary (gumma)	1		1	1			
Neurosyphilis	13	7	6	2	11	8	4
Cardiovascular syphilis	14	3	11	1	13	13	6
History of syphilis	160	50	110	41	119	95	36
"Negative" group	258 (56.8%)	111	147	125	133	136	39
Suspicious history (other venereal disease)	44 (24)	15 (6)	29 (18)	16	28	28	6
History of other venereal disease	78	22	56	42	36	41	12
No history or current lesion	136	74	62	67	69	67	21

All the clinical records were reviewed, and many of the patients were seen personally, by one of us (J.E.C.). On the basis of clinical signs and symptoms and adequate histories, these patients were placed in one of two groups, either among the "syphilitic" or "negative".

From Table 2 it is seen that 196 (43.2 per cent) were considered to be syphilitic and 258 (56.8 per cent) "negative".

Of 7 patients with darkfield-positive primary lesions, 3 gave a reaction with cardiolipin alone, 2 with the complement-fixation technic, and one with both complement-fixation and microflocculation technics; 2 of the 3 later developed positive Kolmer reactions. Four with primary lesions were treated during a previous hospitalization and upon return for follow-up gave reactions with cardiolipin antigens alone. Unfortunately, patients rarely present themselves to the hospital when lesions first develop and it has not been possible to study a large group of persons with untreated early primary syphilis. The 3 patients studied form too small a group from which to draw conclusions but the findings suggest that the cardiolipin tests, being more sensitive, will detect minute quantities of syphilitic reagin. It is interesting to note that in these cases the complement-fixation test was the first to become positive. This might have been expected since in a series of approximately 2000 syphilitic serums this complement-fixation technic has proven to be more sensitive than the microflocculation technic used.[8] One patient having a secondary rash and a battery of strongly positive tests gave only a positive cardiolipin reaction three months after treatment. One patient with a gumma of the nasal septum gave only a minimal cardiolipin reaction on repeated testing.

In 27 persons, some without previous history, clinical features suggestive of cardiovascular and/or central nervous system syphilis were accompanied by positive and weakly positive cardiolipin reactions. Of the 27, 21 had additional specimens and only 10 developed positive Kahn and/or Kolmer tests. One hundred and sixty patients (81.6 per cent) were included in the syphilitic group on the basis of history alone. Only one-third of those with more than one specimen developed reactions with Kahn and/or Kolmer tests. The first knowledge of infection varied from 1 to 45 years previously and in 85 (53.1 per cent) the interval was greater than ten years.

The "negative" group was subdivided into three classes: (a) suggestive but inadequate or unreliable history; (b) history of venereal disease; (c) no history of venereal disease and no current lesion. The second class includes patients who had received penicillin therapy for gonorrhea which may have masked a concomitantly acquired syphilitic infection, permitting the "inactivated" spirochetes to form a less easily detectable lesion elsewhere. Similarly, the widespread use of penicillin for any and all febrile illnesses may temporarily mask syphilis in any stage.

It is realized that a certain number of patients willfully or innocently withhold a syphilitic history. It is, therefore, impossible to determine from history alone how many of the "negative" group may have been infected. It is especially in persons who have no signs or symptoms that knowledge of a previous infection might be of value to the clinician. Some persons have had persistent questioning and re-questioning but have yielded an adequate history only after being confronted with the positive cardiolipin test.

Results of this study have been tabulated to show the incidence of weakly positive and positive reactions, and to evaluate the two technics used. It was found that 170 persons gave weakly positive reactions and 284 positive reactions.

Of the minimal reactors, 26.5 per cent fell into the syphilitic and 73.5 per cent into the "negative" group. One-half of the weakly positive reactions were obtained with the microflocculation technic only. In the "negative" group there was a definite increase in microflocculation reactors (plus-minus, 1 plus) and some increase in total microflocculation reactors (weakly positive and positive) as compared with the syphilitic group. A similar increase was found with the complement-fixation technic in the "negative" group. This would seem to indicate an increase in nonspecific reactions obtained with each technic alone. It is significant, however, that of the 41 persons (weakly positive) included in the syphilitic group on basis of history alone, 27 (65.8 per cent) gave a minimal re-

TABLE 3

ANALYSIS OF CARDIOLIPIN MICROFLOCCULATION AND COMPLEMENT-FIXATION TECHNICS IN POSITIVE AND WEAKLY POSITIVE REACTORS OF SYPHILITIC AND NEGATIVE GROUPS

	NUMBER OF PATIENTS	SYPHILITIC GROUP			"NEGATIVE" GROUP		
		Microflocculation	Complement Fixation	Microflocculation and Complement-Fixation	Microflocculation	Complement-Fixation	Microflocculation and Complement-Fixation
Weakly positive reactors (±, 1+)	170	27 (15.9%)		18 (10.6%)	84 (49.4%)	21 (12.3%)	20 (11.8%)
		45 (26.5%)			125 (73.5%)		
Positive reactors (2+, 3+, 4+)	284	16 (5.6%)	6 (2.1%)	129 (45.4%)	15 (5.3%)	15 (5.3%)	103 (36.3%)
		151 (53.2%)			133 (46.8%)		
Syphilitic group	196	43 (21.9%)	6 (3.1%)	147 (75.0%)			
"Negative" group	258				99 (38.4%)	36 (13.9%)	123 (47.7%)
Total	454	43 (9.5%)	6 (1.3%)	147 (32.4%)	99 (21.7%)	36 (7.9%)	123 (27.1%)
		196 (43.2%)			258 (56.8%)		

activity with the microflocculation technic only. This evidence seems to support the findings of others that another microflocculation technic (Kline) possesses greater sensitivity in latent syphilis,[1, 2, 7, 10] and suggests that these microflocculation reactors may be syphilitics of long standing. Also, the early reaction of the more sensitive complement-fixation test and its increased specificity in malaria as reported by Kent, Boyd, and Sanders[5] suggest that early lesions and relapses have sometimes not been sufficiently considered in the clinical diagnosis.

Over one-half of the positive reactors (stronger than 1 plus) fall into the syphilitic group. The pattern of reactions shows no significant difference in the two groups, and there is a definite increase in proportion of persons reacting with both technics. There were 129 syphilitic patients reacting with both technics; in 104 (80.6 per cent) the complement-fixation test was more sensitive. Simi-

larly, of the 103 "negative" patients who reacted with both technics, 86 (83.5 per cent) reacted more strongly with the complement-fixation technic. Also to be pointed out is the finding that in every serum giving a positive Kahn and/or Kolmer test, reactivity of the same or greater degree was obtained with cardiolipin antigens. Previous reports have indicated, in cases of malaria,[5, 6, 12] a cardiolipin reactivity of lesser degree than obtained with ordinary antigens.

The clinical diagnoses were studied to determine the incidence, in cardiolipin reactors, of diseases commonly known to give nonspecific reactions. In 83 of the 454 patients the diagnosis included one or more of the following: respiratory infections, chronic and acute tonsillitis, other bacterial infections, fever of undetermined origin, other venereal diseases, protozoal infestation (other than malaria), and malignant neoplasm. Forty, or approximately one-half, of these patients were in the syphilitic group. No reactors were found among 28 patients with malaria (with parasitemia) and among a few patients with infectious mononucleosis. This evidence indicates that the over-all pattern of reactions in the syphilitic and negative groups alike is: (a) greater sensitivity of a microflocculation technic in latent syphilis; (b) greater sensitivity of cardiolipin antigens in comparison with ordinary antigens; (c) greater sensitivity of the complement-fixation technic used. Realizing the absence of an appreciable number of patients with diseases commonly known to give false-positive reactions, the authors infer that a high percentage of patients in the "negative" group have had previous syphilitic infection or have current but undetected lesions.

SUMMARY

A total of 60,226 tests was performed on 15,019 serums. Weakly positive or positive cardiolipin reactions and negative Kahn and Kolmer tests were obtained with 991 serums representing 654 persons.

Of 454 patients in whom adequate clinical and social histories were available, 43.2 per cent gave acceptable history of previous syphilitic infection or clinical evidence of a current lesion. It is probable that an additional number of cardiolipin reactors were, or had previously been syphilitic. This latter statement is based on a suspicious clinical history of syphilis, a history of other venereal disease, the low incidence of diseases commonly known to give false positive tests for syphilis, and the similarity of the pattern of reaction in the "negative" group to the syphilitic group.

The authors suggest that the cardiolipin reactivity is produced by increased sensitivity without loss of specificity, and that the routine use of both microflocculation and complement-fixation technics with these antigens is indicated.

Acknowledgments. The authors wish to thank Miss Shirley Kiersky, M.T. (ASCP), and Mr. Benny Jeffus for their excellent assistance. Dr. H. D. Piersma of Lederle Laboratories supplied the cardiolipin and lecithin solutions used in this study.

REFERENCES

1. Andujar, J. J., Anderson, M. M., and Mazurek, E. E.: Cardiolipin blood tests in syphilis. Am. J. Clin. Path., **18**: 199–211, 1948.

2. GIORDIANO, A. S., CULBERTSON, C. S., AND HIGGINBOTHAM, M. W.: Cardiolipin antigens in serologic tests for syphilis. Am. J. Clin. Path., **18**: 193–198, 1948.
3. HARRIS, A., ROSENBERG, A. A., AND RIEDEL, L. M.: A microflocculation test for syphilis using cardiolipin antigen. J. Ven. Dis. Inform., **27**: 169–174, 1946.
4. HARRIS, A., ROSENBERG, A. A., AND DEL VECCHIO, E. R.: The V.D.R.L. slide flocculation test for syphilis. J. Ven. Dis. Inform., **29**: 72–75, 1948.
5. KENT, J. F., BOYD, H. M., AND SANDERS, R. W.: Cardiolipin antigen in the Kolmer complement-fixation test for syphilis. Bull. U. S. Army M. Dept., **8**: 284–293, 1948.
6. KENT, J. F., CULWELL, W. B., COATNEY, G. R., AND COOPER, W. C.: False positive tests for syphilis: A further study of their incidence in sporozoite-induced vivax malaria. J. Lab. and Clin. Med., **33**: 747–752, 1948.
7. KLINE, B. S.: Cardiolipin antigen in the microscopic slide precipitation test for syphilis. Am. J. Clin. Path., **16**: 68–80, 1946.
8. McDEARMAN, S. C., AND KIERSKY, S.: Unpublished data.
9. NEWS AND COMMENT: Cardiolipin antigens in the serdiagnosis of syphilis. Bull. U. S. Army M. Dept., **8**: 247–251, 1948.
10. REIN, C. R.: Serodiagnosis of syphilis; recent advances. J. Michigan State Med. Soc., **46**: 541–549, 1947.
11. REIN, C. R., AND BOSSAK, H. N.: Cardiolipin antigens in the serodiagnosis of syphilis; a microflocculation slide test. Am. J. Syph., Gonor. and Ven. Dis., **30**: 40–46, 1946.
12. REIN, C. R., AND KENT, J. F.: False positive tests for syphilis; study of their incidence in sporozoite-induced vivax malaria. J. A. M. A., **133**: 1001–1003, 1947.
13. STOKES, J. H., BEERMAN, H., AND INGRAHAM, N. R.: Modern Clinical Syphilology. Philadelphia and London: W. B. Saunders Company, pp. 893–966, 1944.
14. STOUT, G. W.: Reactivity of tests using cardiolipin antigens in a limited number of non-syphilitic cases. Am. J. Syph., Gonor. and Ven. Dis., **31**: 314–321, 1947.

STUDIES ON PHAGOCYTOSIS. II*

RUDOLF ALTSCHUL, M.U.Dr., AND AGANETA M. FRIESEN, B.A.

From the Department of Anatomy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

In a previous paper,[2] we reported on phagocytosis of hematoxylin and its loss of color reaction after entering cells. The technic employed was to insert into muscles, catgut threads which had been soaked in hematoxylin solution and subsequently dried. A similar catgut technic was used in the present investigation for studying another aspect of phagocytosis, namely lipophagia.

In some recent work on experimental cholesterol arteriosclerosis we were occupied with the problem of lipophagia and cholesterol phagocytosis. It is not necessary to discuss here the adequacy of the term arteriosclerosis; suffice it to say that some species (rabbits, and to a slighter degree and less regularly, guinea pigs) react to the oral or percutaneous administration of cholesterol with the formation of foam cells and lipoid deposits in blood vessels as well as in other tissues and organs. Rats and cats, however, are refractory. Altschul[1] showed that golden hamsters also are susceptible to cholesterol arteriosclerosis while, according to our recent experiences, prairie gophers are not.

The foam cells of arteriosclerosis are believed to develop from endothelium (Wacker and Hueck[8]), macrophages (Duff[4] and many others), sessile elements of the reticulo-endothelial system (Leary[6]) or from other cell types as well (Hueper[5], Altschul[1]). It is not established with certainty whether such cells become foamy by phagocytosis or whether they undergo fatty degeneration. There is the possibility that the variable susceptibility to lipoidosis in cholesterol arteriosclerosis may be somehow correlated to lipophagia in general and, we have, therefore, undertaken to investigate the latter in those species whose reaction to cholesterol feeding, whether positive or negative, is sufficiently known.

As may be expected, lipophagia has been already studied experimentally, *e.g.*, by Clark and Clark[3] and more recently by Tompkins[7], whose experiments were restricted to cholesterol. However, to our knowledge, the problem has not yet been viewed from the standpoint of species differences. This new approach has little or no relation to former experiments and we, therefore, omit a discussion of the literature.

TECHNIC

Catgut threads were inserted bilaterally into the thigh muscles of rabbits, guinea pigs, golden hamsters, prairie gophers, white rats, and cats, and left there for periods of from 3 to 90 days. Plain catgut was used for some muscle experiments while for some others it was soaked in either a vegetable oil or in a solution of cholesterol in ether with subsequent drying before use in the latter case. A total of 54 animals (16 rabbits, 7 guinea pigs, 4 hamsters, 16 rats, 8

* This work was supported by a grant from the John and Mary R. Markle Foundation.
Received for publication, November 2, 1948.

prairie gophers and 3 cats) were used. After killing the animals, the muscles were excised and fixed in formalin. Frozen sections were stained with Scarlet R (with or without hematoxylin counterstain) or in some cases with osmic acid. Some blocks of the tissue were impregnated with osmic acid or Marchi's fluid, others were embedded in paraffin, sectioned and stained with hematoxylin-eosin. The preparations were then compared with each other with reference to the number and size of lipophages in the granulation tissue and the time of their appearance.

RESULTS AND COMMENTS

In some cases, plain catgut had been inserted in one leg and catgut of the same gauge, soaked in either vegetable oil or cholesterol solution, in the other leg of the same animal and left there for the same period, but there was very little if any difference between the results in either leg.

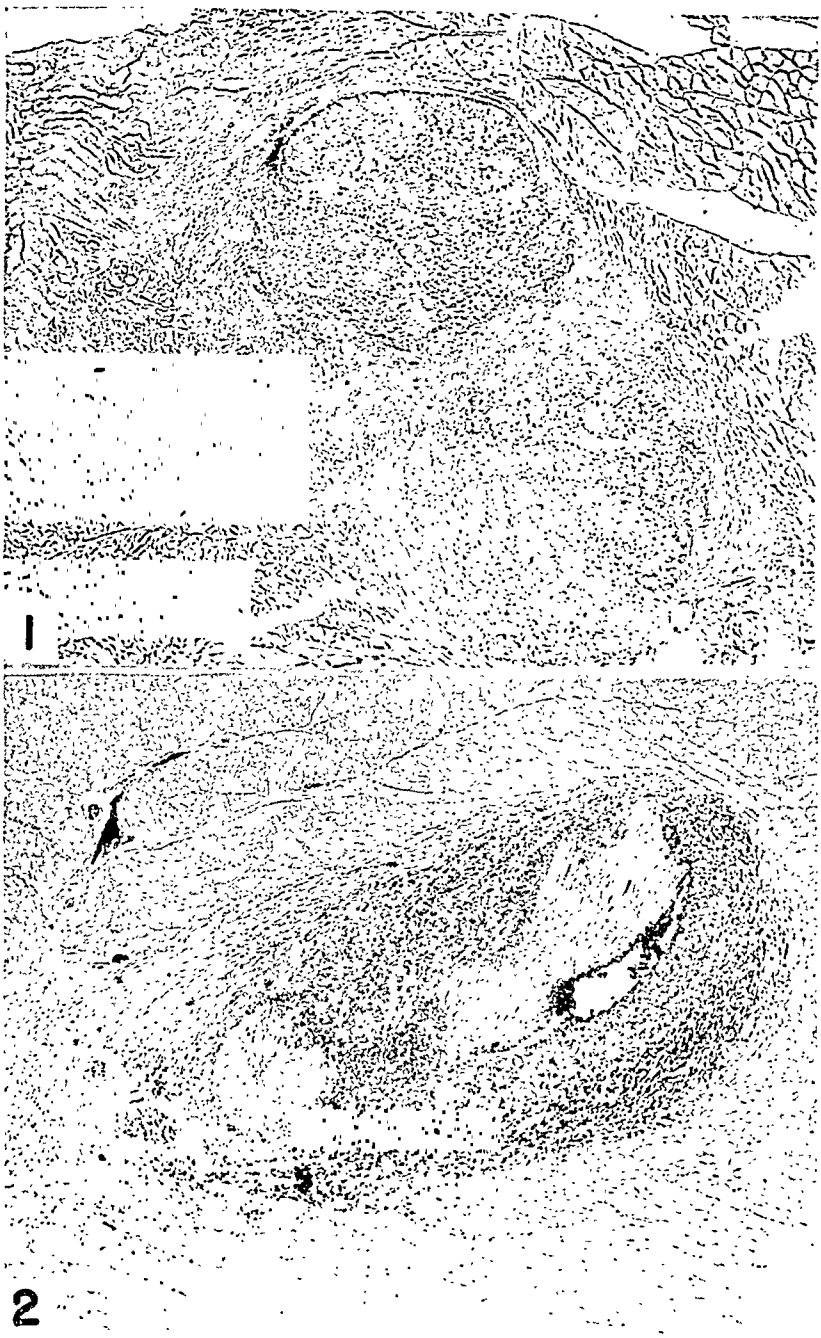
According to Clark and Clark,[3] phagocytosis of fat begins a few hours after its introduction into the tissues, while Tompkins[7] observed cholesterol phagocytosis after several minutes. In this early period of our experiments there would have been too large a margin of error in making a comparison of the number and size of lipophages with the methods we used because the granulation tissue was not well developed, and there were thus too few lipophages to observe. We found that the time of appearance of the multitude of lipophages was not the same in the various species of animals used.

It was deemed necessary to consider also the food intake of the animals in relation to lipophagia. Therefore, a special experiment was carried out with rats, some of which were fed plenty of fat and carbohydrates while others from the same stock were kept on a near-starvation diet. The lipophagia in the granulation tissue around the catgut presented no definite difference between the two groups.

Comparison of the intensity of lipophagia in the various animals allowed us to draw up an intensity scale which lists the species of animals in order of decreasing intensity of lipophagia exhibited as follows: rat, prairie gopher, golden hamster, cat, rabbit, guinea pig. The order of cat and rabbit was difficult to decide and should be taken with some reserve.

It is reasonable to argue that we did not observe differences in potential lipophagic activity but a quantitative difference in fatty degeneration of muscle tissue which induced a lesser or greater degree of lipophagia. However, fatty degeneration of skeletal muscle fibers was severe in the guinea pigs and yet the lipophagia was scanty.

On the other hand, previous experiments with cholesterol feeding allow us to arrange the species as to their readiness to react with lipoidosis and the following declining scale is obtained: rabbit, golden hamster, guinea pig, prairie gopher, cat, rat. The cat and rat are refractory to cholesterol lipoidosis, with no difference between the two species. In the prairie gopher no lipoidosis appears with the exception of occasional formation of cholesterol gall stones.



FIGS. 1 AND 2. Photomicrographs of frozen sections of skeletal muscle into which plain catgut has been inserted ten days before death. Scarlet R with no counterstain. × 29. Note the intensity of lipophagia shown by the darkly stained cuff of lipophages in Figure 2 (from rat), in contrast to the paucity of lipophagia in Figure 1 (from rabbit).

A comparison of the scales for local lipophagia and experimental cholesterol lipoidosis shows that there is no parallel between the results of the two experimental approaches.

SUMMARY

An experiment has been described which indicates that lipophagia in granulation tissue of skeletal muscle varies in different species as to time of onset and intensity. Previous experiments have shown that the intensity of cholesterol lipoidosis in experimental arteriosclerosis also varies according to the species used. However, no strict relationship is evident between intensity of local lipophagia in damaged skeletal muscle and intensity of cholesterol lipoidosis.

REFERENCES

1. ALTSCHUL, R.: Experimental cholesterol arteriosclerosis. Arch. Path., **42**: 277–284, 1946.
2. ALTSCHUL, R., AND FRIESEN, A. M.: Studies on phagocytosis. Am. J. Clin. Path., **17**: 444–448, 1947.
3. CLARK, E. R., AND CLARK, E. L.: Observations on changes in blood vascular endothelium in the living animal. Am. J. Anat., **57**: 385–438, 1935.
4. DUFF, G. L.: Experimental cholesterol arteriosclerosis and its relationship to human arteriosclerosis. Arch. Path., **20**: 81–123; 259–304, 1935.
5. HUEPER, W. C.: Macromolecular substances as pathogenic agents. Arch. Path., **33**: 267–290, 1942.
6. LEARY, T.: Genesis of atherosclerosis. Arch. Path., **32**: 507–555, 1941.
7. TOMPKINS, E. H.: Reaction of the reticuloendothelial cells to subcutaneous injections of cholesterol. Arch. Path., **42**: 299–319, 1946.
8. WACKER, L., AND HUECK, W.: Ueber experimentelle Atherosklerose und Cholesterinaemie. München. med. Wchnschr., **60**: 2097–2101, 1913.

PHOSPHATASES IN BLOOD OF MAN

Values in Whole Blood, Plasma, Cytolysates and Erythrocytic Suspensions*

H. BEHRENDT, M.D.

From the Department of Pediatrics, New York Post-Graduate Medical School and Hospital, New York, New York

The results of previously reported observations[2] indicate that human erythrocytes contain primarily "acid" phosphomonoesterase with an optimal activity at pH 4.9–5.0, while "alkaline" phosphatase, with an optimum at 9.0, is present only in very small amounts. The reverse is true of normal human plasma, in which alkaline phosphatase activity is high as compared with acid phosphatase activity. The predominance of acid phosphatase activity in red cells and of alkaline phosphatase activity in plasma can be demonstrated with either glycerophosphate or phenylphosphate serving as substrate. The measurements made by King, Wood, and Delory[15] confirmed these findings in principle. The absence of a magnesium effect and the weakness of fluoride inhibition, as established for the red cell phosphatase at pH 4.9, corroborated the suggestion made by Sullivan, Gutman, and Gutman[21] that the acid enzymes of red cells and plasma are not identical.

All estimations of phosphatase activity of erythrocytes reported so far were carried out on washed and subsequently hemolyzed red cells. The studies to be reported here were made on whole blood, blood plasma, blood cytolysates (hemolyzed erythrocytes), and suspensions of erythrocytes in saline solution. With few exceptions, measurements were made only at the established optimum pH of the acid and alkaline enzymes.

PLASMA AND CYTOLYSATES

Venous blood was withdrawn into tubes containing enough dried sodium oxalate solution or heparin to prevent clotting. A portion of this blood sample was transferred into a hematocrit tube for estimation of the relative cell volume. In the remaining portion, plasma and cells were separated by centrifugation; as much plasma as possible was pipetted off and the remaining red cells were washed three times with physiologic saline solution. The washed cells obtained from A ml. of whole blood were hemolyzed by adding distilled water to bring the total volume of the cytolysate to A ml., or to $1.5 \times A$ ml., designated as dilutions 1 and 1.5, respectively.

A. Measurements with Glycerophosphate as Substrate

According to the principles of the Bodansky method,[3] plasma or cytolysate was brought to act upon β-glycerophosphate, using veronal buffer[17] of pH 8.9 to maintain the optimal alkaline reaction, and veronal-acetate buffers[18] to study the acid phosphatase activity at pH 4.9.

* Received for publication, November 22, 1948.

Example: 0.4 ml. plasma or cytolysate
0.5 ml. 1/20 M disodium glycerophosphate
2.0 ml. 5 per cent monosodium diethylbarbiturate
2.1 ml. distilled water.

These blood-substrate-buffer mixtures were incubated at 37 C. for one hour. After addition of 3 ml. of 20 per cent trichloracetic acid and subsequent filtration, phosphorus was determined in an aliquot of the clear filtrate according to the method of Fiske and SubbaRow.[6] A control was set up and run in the same way, except that the trichloracetic acid was added prior to the incubation. The difference between the phosphorus content of sample and control represents the phosphatase activity of the blood derivative. The results are expressed as units of phosphatase activity, one unit being equivalent to one

TABLE 1

PHOSPHATASE ACTIVITY OF HUMAN PLASMA AND LAKED ERYTHROCYTES, USING SUBSTRATE β-GLYCEROPHOSPHATE AND BUFFERS VERONAL AT pH 8.9 AND VERONAL-ACETATE AT pH 6.1 AND 4.9

EXPERIMENT NO.	AGE OF BLOOD DONORS	ANTICOAGULANT	HEMATOCRIT, PER CENT	pH OF BUFFER-SUBSTRATE	PHOSPHATASE ACTIVITY, UNITS		
					In Plasma	In Cytolysate	In Erythrocytes
52	49 yr.	Oxalate	40	8.9	1.1	0.32	1.2
				4.9	0.2	0.67	2.5
53	32 yr.	Oxalate	41	8.9	7.1	0.6	2.2
				4.9	0.3	1.0	3.7
63	13 mo.	Heparin	39	8.9	4.8	0	—
64[a]	18 mo.	Heparin	25	8.9	2.7	0.2	1.2
				4.9	0.1	0.7	4.2
65[b]	18 mo.	Heparin	21	8.9	0.9	1.0	7.1
				6.1	0.6	1.1	7.7
				4.9	0.1	3.9	27.9
73a[c]	6 mo.	Heparin	33	8.9	19.6	0.6	2.7
				4.9	4.0	0.9	4.1
74a[d]	47 yr.	Oxalate	44	8.9	11.8	0	—
				4.9	0.3	0.9	3.0

Diagnosis: [a] sickle cell anemia, [b] toxic anemia, [c] biliary obstruction, [d] Paget's disease.

milligram of phosphorus liberated per 100 ml. of plasma or cytolysate during one hour of incubation. The phosphatase activity of erythrocytes was calculated as follows:

$$\text{Phosphatase units in erythrocytes} = \text{phosphatase units in cytolysate} \times \frac{100 \times D}{V}$$

where D = dilution of cytolysate
V = relative volume of red cells in whole blood.

As shown in Table 1, phosphatase activity is always present at pH 4.9, both in plasma and cytolysate. In this acid medium, cytolysates at all times exhibit greater activity than plasma. At pH 8.9, however, cytolysates show little, if any, activity. Figure 1 summarizes the results obtained on normal human blood (No. 52, 53, 63 of Table 1). On the whole, the results of these experiments with glycerophosphate as substrate are not very impressive as far as the enzymic action of cytolysates is concerned. The amount of phosphorus liberated from the substrate by the acid and the alkaline phosphatases of cytolysates represents

only fractions of the phosphorus concentrations liberated by the alkaline plasma phosphatase. The outcome of experiments using phenylphosphate, however, is more enlightening.

TABLE 2

PHOSPHATASE ACTIVITY OF HUMAN PLASMA AND LAKED ERYTHROCYTES IN BLOOD OF NORMAL COMPOSITION, USING SUBSTRATE SODIUM PHENYLPHOSPHATE AND BUFFER VERONAL-ACETATE, DILUTION FACTOR OF CYTOLYSATES 1.5 AND HEPARIN AS ANTICOAGULANT

EXPERIMENT NO.	AGE OF BLOOD DONOR IN YEARS	HEMATOCRIT, PER CENT	pH OF BUFFER-SUBSTRATE	PHOSPHATASE ACTIVITY, UNITS		
				In Plasma	In Cytolysate	In Erythrocytes
67	2	38	8.9	9.2	0.2	0.8
			6.1	0.4	6.7	26.5
			4.9	0.6	23.9	94.4
68	6	38	8.9	6.3	1.2	4.7
			6.1	3.3	16.7	66.0
			4.9	3.6	21.7	85.7
			3.8	2.8	14.8	58.5
71	49	41	8.9	5.3	1.3	4.8
			6.1	2.4	21.0	76.9
			4.9	2.4	26.0	95.1
			3.8	3.1	15.4	56.4
72	12	38	8.9	5.0	1.0	4.0
			7.6	—	3.1	12.2
			6.1	2.1	21.5	85.0
			4.9	—	29.0	114.6
			3.8	2.1	24.0	94.8
			2.6	—	9.2	36.3
76	2	39	8.9	10.3	1.3	5.0
			6.1	—	58.1	223.7
			4.9	9.9	68.7	264.5
78	3	38	8.9	8.8	0.5	2.0
			4.9	7.3	77.3	305.3
79	49	43	4.9	0.7	53.3	185.9
82	5	40	8.9	11.8	0	0
			4.9	5.3	50.9[a]	127.3

[a] Dilution factor 1.0.

B. Measurements with Phenylphosphate as Substrate

Plasma or cytolysate obtained as described above was added to the buffered substrate consisting of 1/200 M sodium phenylphosphate and Michaelis' veronal-acetate buffers[18].

Example: 0.5 ml. plasma or cytolysate
5.0 ml. buffer
4.5 ml. sodium phenylphosphate.

The mixture was incubated at 37 C. for one hour, 4.5 ml. of diluted phenol reagent was added, and the phenol liberated was determined by the method of Gutman and Gutman.[5] A control was treated similarly, except that the phenol reagent was added prior to in-

cubation. The difference in the amount of phenol content between test sample and control gave the phosphatase activity of the sample. The results were expressed as units of phosphatase activity, one unit being equivalent to that amount of enzyme which will liberate 1 mg. of phenol per 100 ml. of blood derivative from the phenylphosphate substrate during one hour. The phosphatase content (in units) of the erythrocytes was calculated as described above.

The results of 13 such experiments are given in Tables 2 and 3. In principle, they confirm the findings obtained with glycerophosphate, but make the action of the acid enzyme appear more definite. When the activity of cytolysates, as measured in these experiments, is expressed in units of red cell phosphatase and compared with the amount of plasma phosphatase units from the same blood

TABLE 3

PHOSPHATASE ACTIVITY OF HUMAN PLASMA AND LAKED ERYTHROCYTES IN BLOOD OF ABNORMAL COMPOSITION, USING SAME SUBSTRATE, BUFFERS, DILUTION FACTOR OF CYTOLYSATES, AND ANTICOAGULANT AS IN TABLE 2

EXPERIMENT NO.	DIAGNOSIS	AGE OF BLOOD DONOR	HEMATOCRIT, PER CENT	pH OF BUFFER-SUBSTRATE	PHOSPHATASE ACTIVITY, UNITS		
					In Plasma	In Cytolysate	In Erythrocytes
73b	Biliary obstruction	6 mo.	33	8.9	28.1	1.2	5.5
				4.9	9.4	41.5	188.6
74b	Paget's disease	47 yr.	44	8.9	12.9	2.9	10.0
				6.1	—	36.2	123.4
				4.9	7.7	58.2	198.5
75	Biliary obstruction	12 yr.	32	8.9	45.5	9.5	44.5
				6.1	—	64.7	303.3
				4.9	5.3	78.2	366.6
77	Prostatic cancer with metastases	56 yr.	40	8.9	5.4	—	—
				6.1	—	51.8	194.3
				4.9	20.6	66.2	248.3
83	Sarcoma of femur	10 yr.	39	8.9	34.5	1.1	1.4
				4.9	7.8	49.6	190.8

sample, the following relationship appears to be typical for human blood of any age group and of normal composition (Table 2 and Fig. 2): Phosphatase activity at pH 8.9 is more than twice as great in the plasma as in the red cells, whereas at pH 4.9 erythrocytes possess an activity at least a hundredfold that of plasma. The values of acid red cell phosphatase range between 94.4 and 305.3 units, with an average of 159.1, while King and his collaborators[15] found somewhat higher figures, namely, a range from 200 to 400 units, with an average of 340.

A second group of determinations (Table 3) was performed on blood samples from patients whose diseases affected the relative red cell volume or the activity of alkaline or acid plasma phosphatase. In these abnormal specimens, the acid phosphatase of the red cells was found to be higher than in cells of normal blood, averaging 238.4 units, with a range between 188.6 and 366.6 units. The number of estimations in each group of diseases is too small to permit any conclusions as to the significance of such an apparent increase.

CYTOLYSATES, RED CELL SUSPENSIONS, AND WHOLE BLOOD

An occasional experiment revealed that the hydrolytic effect of whole blood upon buffered phenylphosphate was almost as strong as that of laked erythrocytes when the blood was prevented from being hemolyzed during the incubation period. This observation prompted the investigation of the phosphatase activity of nonhemolyzed red cells under varying conditions.

To insure preservation of their physical integrity during the period of enzymic action, erythrocytes were brought to act upon the substrate as follows: X ml. of heparinized blood were centrifuged and the plasma removed. The remaining cells were washed 3 times

TABLE 4

PHOSPHATASE ACTIVITY OF LAKED AND SUSPENDED HUMAN ERYTHROCYTES IN BLOOD OF NORMAL COMPOSITION USING SUBSTRATE ISOTONIC SODIUM PHENYLPHOSPHATE, AND BUFFER ISOTONIC VERONAL-ACETATE-SODIUM CHLORIDE AT pH 4.9

EXPERIMENT NO.	HEMATOCRIT, PER CENT	PHOSPHATASE ACTIVITY, UNITS					
		In Cytolysate	In Suspension			In Erythrocytes	
		Dilution 1.5	Dilution			Calculated from	
			1.0	1.5	3.0	Cytolysate	Suspension
92	41	—		43.8		—	160.2
99	42	—			26.3	—	187.9
71	41	26.0		23.8		95.1	105.4
72	38	29.0		34.0		114.6	134.2
83[a]	39	49.6		38.4		190.8	147.7
84	43	12.5	45.3			43.6	105.4
85	41	11.4		30.3		40.7	110.9
91	42	13.6	44.5			48.6	106.0
94	41	25.7		24.0		94.0	87.8
95	41	72.9		60.7		266.7	246.5
96[b]	34	35.7		28.4		157.5	125.3

Diagnosis: [a] sarcoma of femur, [b] sickle cell anemia.

with physiologic saline solution and then resuspended in enough saline to bring the volume of the suspension up to the original sample volume of X ml., or to 1.5 times X ml., or to 3 times X ml. The dilutions were designated as 1, 1.5, and 3, respectively. In portions of these suspensions, the phosphatase was estimated under isotonic conditions, using an isotonic substrate-buffer mixture of pH 4.9 consisting of 0.5 parts 0.107 M (2.34 per cent) disodium phenylphosphate, and 9.5 parts of Michaelis' isotonic veronal-acetate buffers.[18] A hematocrit determination was made on a separate blood sample.

Example: 0.5 ml. blood derivative (red cell suspension)
0.5 ml. isotonic phenylphosphate
9.5 ml. isotonic buffer solution.

The reaction mixture was almost always free of hemolysis at the end of the incubation period, giving proof of the isotonicity of the set-up. Mixtures showing traces of hemolysis were discarded. The incubation and colorimetric determination of the liberated phenol and the calculation of phosphatase activity as units were carried out as described above for the cytolysates. For the comparative study of cytolysates, the same isotonic substrate-buffer mixture was employed as for the red cell suspensions.

After 2 preliminary measurements (No. 92 and 99 in Table 4) had shown that red cell suspensions exert a strong enzymic activity upon phenylphosphate, comparative determinations were made on cytolysates and suspensions derived from the same blood sample. The results are recorded in Table 4. As may be seen from the 2 last columns of this table, in 8 of 9 cases the erythrocyte suspensions exerted phosphatase activity at least as strong as that of the hemolyzed cells. In one experiment (No. 83), the activity of the suspensions was slightly less than that of the cytolysate, but in 6 instances (No. 71, 72, 84, 91, 96) the action of the suspension was superior to that of the cytolysate.

If the outcome of these tests may be taken as sound evidence, it would appear that the phosphatase principle, when retained within the red cell membrane, produces an equal or even stronger enzymic action upon the extracellular substrate than the same enzyme when released by cytolysis into the substrate-buffer

TABLE 5

PHOSPHATASE ACTIVITY OF HUMAN ERYTHROCYTES SUSPENDED IN VARIOUS MEDIUMS IN BLOOD OF NORMAL COMPOSITION USING SUBSTRATE ISOTONIC SODIUM PHENYLPHOSPHATE, BUFFER ISOTONIC VERONAL-ACETATE-SODIUM CHLORIDE AT PH 4.9 AND ANTICOAGULANT HEPARIN

EXPERIMENT NO.	PHOSPHATASE ACTIVITY, UNITS					
	In Plasma	In Whole Blood	In Cell Suspension in Saline, Dilution Factor 1.0	In Cell Suspension + Plasma, in Proportion		
				1:1	1:2	1:3
84	1.0	22.8	45.3	—	—	—
85	1.0	11.6	45.5	—	—	—
92	—	—	65.7	4.5	—	0
94	0.5	27.2	36.0	27.9	1.7	—
95	1.9	61.5	91.1	60.0	—	—
96[a]	3.3	35.9	42.6	40.5		

[a] Diagnosis, sickle cell anemia.

medium. Some further evidence may be of help in explaining this finding. No phosphatase activity could be ascertained in pooled samples of the saline solutions used for washing the red cells. Negative results were also obtained with the supernatant liquid of a mixture consisting of 0.5 ml. of red cell suspension and 9.5 ml. of isotonic buffer solution, when tested for phosphatase after incubation for one hour.* It may be concluded that neither during the process of washing nor during the period of incubation does the active principle penetrate through the red cell membrane into the extracellular phase of the suspension.

WHOLE BLOOD AND ERYTHROCYTE SUSPENSIONS

As a result of these findings, it seemed desirable to test the phosphatase activity of whole blood, *i.e.*, of red cells suspended in plasma. Employing the same isotonic arrangement as before, comparative estimations were made on

* These tests were made upon the suggestion of Dr. L. Michaelis of New York.

plasma, whole blood, and erythrocytes suspended in saline solution, all prepared from the same blood specimen. The findings are listed in Table 5. When tested at pH 4.9, whole blood was found to be consistently less active than the red cell suspension containing a similar (percentile) volume of erythrocytes.

TABLE 6

EFFECT OF PLASMA ON THE PHOSPHATASE ACTIVITY OF RED CELL CYTOLYSATES IN NORMAL HUMAN BLOOD, USING SUBSTRATE SODIUM PHENYLPHOSPHATE AND BUFFER VERONAL-ACETATE AT pH 4.9

EXPERIMENT NO.	PHOSPHATASE ACTIVITY, UNITS		
	Cytolysate, Dilution 1.0	Cytolysate + Plasma, in Proportion	
		1:1	1:2
94	38.6	4.1	—
95	109.4	30.5	2.1
97	83.9	19.5	—

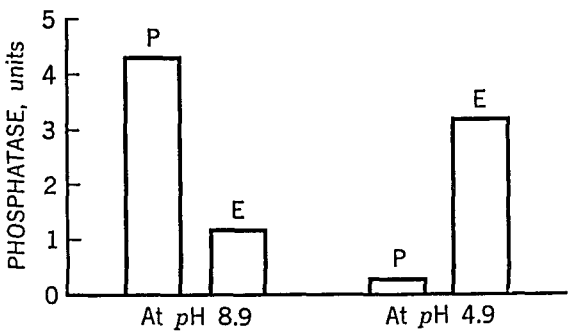


FIG. 1

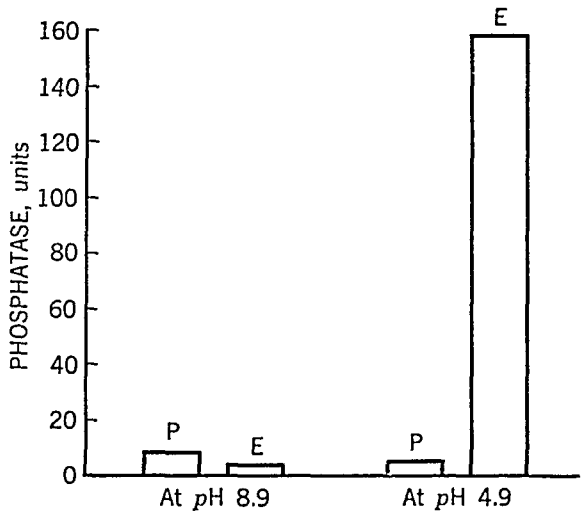


FIG. 2

FIG. 1. Units of phosphatase activity of human plasma (P) and erythrocytes (E) in substrate β-glycerophosphate. Values represent averages of experiments No. 52, 53 and 63 from Table 1.
FIG. 2. Units of phosphatase activity of human plasma (P) and erythrocytes (E) in substrate phenylphosphate. Values represent averages from Table 3.

In other words, the presence of plasma in the medium surrounding the intact cells seems to interfere with the enzymic action of the intracellular phosphatase upon the phenylphosphate in the extracellular medium.

If this assumption is correct, the activity of red cells suspended in saline solution will be reduced by the addition of plasma at the start of the incubation period. This was tested in four experiments.

Example: 0.25 ml. red cell suspension
0.5 ml. isotonic phenylphosphate
9.5 ml. isotonic veronal-acetate buffer
+0.25 ml. plasma.

Table 5 reveals that addition of plasma did inhibit the phosphatase activity of cell suspensions in all instances. When the proportion of plasma to suspension was 1:1, phosphatase activity was reduced to approximately that of whole blood in 3 of the 4 tests. When the ratio of plasma to suspension was further increased (2:1 and 3:1) the phosphatase activity of the suspended erythrocytes declined sharply or disappeared completely. The actual concentration of plasma in the incubated mixture varied from 2.4 to 6.8 per cent in the different tests.

This inhibitory action of plasma, however, can also be demonstrated on solutions of laked red cells tested in the non-isotonic set-up described above. Table 6 shows that the inhibition by plasma of the phosphatase contained in cytolysates was strong or almost complete, depending upon the ratio of plasma to cytolysate in the substrate-buffer mixture.

COMMENT AND SUMMARY

The following acid phosphatases have been found in human blood since Folley and Kay[7] presented their classification of the various phosphatases:

(1). The serum acid phosphatase occurring in normal blood, with an optimal activity at pH 4.9–5.0.[9] Its action is markedly inhibited by sodium fluoride and citrate, and not accelerated by magnesium chloride. This enzyme splits α-glycerophosphate with no greater speed than β-glycerophosphate, while the scission of phenylphosphate is most easily catalyzed. These properties coincide with those of the acid phosphatases present in liver, spleen, kidney and bone, organs assumed to be the source of this normal serum acid phosphatase.[21]

(2). The serum acid phosphatase appearing in the plasma of patients with metastasizing cancer of the prostate.[1,10] Its source is the prostatic cancerous tissue.[11] In some respects, the enzyme resembles the normal serum acid phosphatase[2]: optimal activity at pH 4.9 and inhibition by fluoride; it is, however, also inhibited by alcohols, for instance ethyl alcohol,[13,21] and is little affected by citrate.[21]

(3). The erythrocyte acid phosphatase, with an optimal pH at 4.9, present in cells of persons of all ages.[2, 15] While this enzyme, according to some observers,[2, 12] is not significantly affected by fluoride or magnesium, an inhibition by both ions has been found by others.[15] The action of this enzyme upon phenylphosphate is more than 100 times greater than that of the normal serum acid phosphatase. The phosphatases occurring in the erythrocytes of horse, rabbit, and guinea pig differ from the acid phosphatase of red cells of man in various respects, chiefly in their optimal pH.[19, 20]

While the properties of human red cell phosphatases and the kinetics of the reactions they catalyze, have so far been investigated on laked whole blood or laked erythrocytes, the present report includes a study of the intracellular

phosphatase in living cells. By using an isotonic set-up of cell suspensions, buffer and substrate, an attempt has been made to study *in vitro* the factors governing the substrate-enzyme accessibility *in vivo*. From these first observations, it would seem that at pH 4.9 phenylphosphate must penetrate through the erythrocyte membrane in order to be acted upon by the enzyme. This would resemble the penetration of such permeants as phenylacetate, *i.e.*, the introduction of a phenyl in the place of a hydrogen, favoring the lipoid solubility of the permeant. However, unlike phenylacetate, phenylphosphate has no hemolytic effect, probably because of its ionization.* It should not be difficult to supply experimental proof of such a permeation. The measurement of anhydrase activity within living, suspended red blood cells by Booth[4] and by Keilin and Mann[14] confirms the potential significance of quantitative enzyme studies on suspended red corpuscles. The isotonic arrangement would also permit comparison of the kinetics of phosphatase reactions catalyzed by intact living erythrocytes and by red cell cytolysates. Obviously, the discrepancy between fermentation by yeast cells and by yeast extracts[5, 16] invites speculation on such a comparison. However, final conclusions as to the activity of "cytolysates" as employed in the present studies should await the results of other experiments in which the activity of leukocytes and platelets has been properly taken into account.

CONCLUSIONS

Our observations indicate that at pH 4.9, phenylphosphate must penetrate through the erythrocyte membrane in order to be acted upon by the enzyme, phosphatase.

Acid phosphatase activity of red cells is reduced in the presence of plasma. This inhibition is equally strong when suspensions or cytolysates are employed. Apparently, the action of plasma upon the enzyme is not mediated by osmotic factors bound to the presence of intact cells.

REFERENCES

1. Barringer, B. S., and Woodard, H. Q.: Prostatic carcinoma with extensive intraprostatic calcification, with discussion of possible role to prostatic phosphatase. Tr. Am. A. Genito-Urin. Surgeons, **31**: 363–369, 1938.
2. Behrendt, H.: Phosphatase activity of human erythrocytes. Proc. Soc. Exper. Biol. and Med., **54**: 268–270, 1943.
3. Bodansky, A.: Notes on determination of serum inorganic phosphate and of serum phosphatase activity. Am. J. Clin. Path., Tech. Suppl., **7**: 51–59, 1937.
4. Booth, V. H.: Carbonic anhydrase activity inside corpuscles. Enzyme-substrate accessibility factors. J. Physiol., **93**: 117–128, 1938.
5. Brooks, S. C.: Permeability and enzyme reactions. In: Advances in Enzymology. Vol. 7, New York: Interscience, pp. 1–34, 1947.
6. Fiske, C. H., and SubbaRow, V.: The colorimetric determination of phosphorus. J. Biol. Chem., **66**: 375–400, 1925.
7. Folley, S. J., and Kay, H. D.: The phosphatases. Ergebn. d. Enzymforsch., **5**: 159–212, 1936.
8. Gutman, A. B., and Gutman, E. B.: "Acid" phosphatase activity of serum of normal human subjects. Proc. Soc. Exper. Biol. and Med., **38**: 470–473, 1938.
9. Gutman, A. B., and Gutman, E. B.: "Acid" phosphatase occurring in serum of patients with metastasizing carcinoma of prostate gland. J. Clin. Investigation, **17**: 473–478, 1938.

* I am indebted to Dr. S. C. Brooks, Berkeley, Calif., for formulating these conjectures.

10. GUTMAN, E. B., AND GUTMAN, A. B.: Estimation of "acid" phosphatase activity of blood serum. J. Biol. Chem., **136:** 201–209, 1940.
11. GUTMAN, E. B., SPROUL, E. E., AND GUTMAN, A. B.: Significance of increased phosphatase activity of bone at the site of osteoplastic metastases secondary to carcinoma of prostate gland. Am. J. Cancer, **28:** 485–495, 1936.
12. GUTMAN, E. B., AND GUTMAN, A. B.: Erythrocyte phosphatase activity in hemolyzed sera and estimation of serum "acid" phosphatases. Proc. Soc. Exper. Biol. and Med., **47:** 513–515, 1941.
13. HERBERT, F. K.: The differentiation between prostatic phosphatase and other acid phosphatases in pathological human sera. Biochem. J., **39:** IV, 1945.
14. KEILIN, D., AND MANN, T.: Activity of carbonic anhydrase within red blood corpuscles. Nature, London, **148:** 493–496, 1941.
15. KING, E. J., WOOD, E. J., AND DELORY, G. E.: Acid phosphatase of the red cells. Biochem. J., **39:** XXIV–XXV, 1945.
16. MEYERHOF, O.: The origin of the reaction of Harden and Young in cell-free alcoholic fermentation. J. Biol. Chem., **157:** 105–119, 1945.
17. MICHAELIS, L.: Diethylbarbiturate buffer. J. Biol. Chem., **87:** 33–35, 1930.
18. MICHAELIS, L.: Der Acetat-Veronal-Puffer. Biochem. Ztschr., **234:** 139–141, 1931.
19. ROCHE, J.: Blood phosphatases. Biochem. J., **25:** 1724–1733, 1931.
20. ROCHE, J., AND BULLINGER, E.: Sur le système phosphatasique des hématies. Enzymologia, **7:** 278–291, 1939.
21. SULLIVAN, T. J., GUTMAN, E. B., AND GUTMAN, A. B.: Theory and application of the serum "acid" phosphatase determination in metastasizing prostatic carcinoma; early effects of castration. J. Urol., **48:** 426–458, 1942.

CLINICOPATHOLOGIC CONFERENCE*

GRANVILLE A. BENNETT, M.D., AND JEROME T. PAUL, M.D.

From the University of Illinois College of Medicine, Chicago, Illinois

CLINICAL DATA

A 31 year old American-born woman of Japanese parentage was admitted to this hospital complaining of (1) swelling of the legs for 5 or 6 months, (2) pain in the knees, ankles and wrists, intermittently for 2 years, (3) patchy red rash over the cheeks and bridge of the nose for $2\frac{1}{2}$ years and (4) urinary frequency with pain for 4 years.

Past history. The patient was in fairly good health until $2\frac{1}{2}$ years before admission when a patchy red rash appeared on the cheeks and over the bridge of the nose. This lasted for about 6 months when it disappeared following an attack of measles. Approximately one month after recovering from the measles she became jaundiced. Eight years before admission the patient had beriberi in Japan. In the same year an operation was performed and she was told that her appendix, tubes, ovaries and a part of the womb were removed.

Physical examination was essentially negative except for slight brawny edema of the lower legs. The skin over the cheeks, arms and legs felt thicker and less pliable than normal. The temperature was 98 F., pulse rate 62, respiratory rate 18, blood pressure 124/72. The heart was of normal dimensions, the rhythm was regular and no murmurs were heard. The lungs were clear to percussion and auscultation. The abdomen was negative.

Laboratory findings were as follows: Hemoglobin 9.6 to 14.5 Gm.; erythrocyte counts 3.45 to 4.23 millions, leukocyte counts 4100 to 6200, with normal differentials; sedimentation rate (corrected) on two occasions 45 mm. in 1 hour; urine, specific gravity 1.006 to 1.022, a few hyaline casts and a few pus cells, albumin 3 plus, three quantitative albumin tests, 0.5 Gm. per liter; blood glucose 85, nonprotein nitrogen 33, uric acid 3.5, CO_2 combining power 61 volumes per cent, cholesterol 378, esters 245, sodium chloride 512, (1) serum albumin 2.1 Gm., globulin 2.8 Gm., (2) serum albumin 2.4, serum globulin 3.3; urea clearance 13.5 ml. per min. standard; basal metabolic rate plus 2 per cent; Congo red test 67 per cent excretion; Wassermann and Kahn tests negative.

Roentgenograms of the chest were negative. After $2\frac{1}{2}$ months in the hospital the patient was discharged to her home.

Second admission. Two months later the patient returned to the hospital because of a recurrence of pain in the joints (knees, shoulders and thumbs) of 5 days' duration and nausea of 2 days' duration. The temperature was 97.8 F., pulse rate 88, respiratory rate 20, blood pressure 115/65. Except for the presence of a small amount of fluid in the right knee joint and increased limitation of motion of both knees and shoulders, the physical findings were unchanged from those of the previous examination. The laboratory findings showed hemo-

* Received for publication, October 28, 1948.

globin 12.5 Gm., erythrocyte count 3.87 millions, leukocytes 6950, neutrophils 78, monocytes 14, lymphocytes 7, eosinophils 1; urine, specific gravity 1.020, albumin 3 plus, sediment contained a few red cells, a few white cells and a few granular casts; blood glucose 86, nonprotein nitrogen 36, CO_2 combining power 44, serum albumin 2.0 Gm., globulin 3.6 Gm.

Clinical course. The patient had some nausea and vomiting during the first few days in the hospital, but was otherwise well. On the 11th hospital day some violaceous discoid lesions appeared on both cheeks. The urinary output diminished at this time. She was given plasma, Amigen and forced feedings by tube of a high protein food mixture. Two weeks later it was noted that the dermal lesions on the face were more numerous and prominent and that a similar lesion had appeared on the left breast. Examination of the heart and lungs was negative at this time and there was no edema. A few days later a spiking fever developed and two urine specimens (catheterized) revealed gram-negative rods. Streptomycin therapy was given and the fever subsided somewhat.

On the 37th day of hospitalization crepitant rales were heard over the lung bases and a soft systolic murmur was noted at the cardiac apex. The second sound at the apex was reduplicated. The face appeared puffy but there was no sacral or ankle edema. The following day the carbon dioxide combining power was 29 volumes per cent, the nonprotein nitrogen was 66 mg. and the chlorides 577 mg., and shifting dullness was found in the flanks. Two days prior to death (43rd day of hospitalization) the patient had a convulsion lasting about 30 seconds.

DISCUSSION

Dr. J. T. Paul, Department of Medicine. "On admission, the complaints presented by this 31 year old Japanese woman bring up several interesting points. She had had pain in the knees, ankles and wrists intermittently for 2 years and a patchy red rash over the cheeks and bridge of the nose for $2\frac{1}{2}$ years. These symptoms and signs are particularly important in that they immediately give some clue to the possible underlying problem. As you know, erythema multiforme is in many instances closely related to or a part of an acute rheumatic fever. The onset of disseminated lupus erythematosus is often characterized by an arthralgia and an erythematous rash involving the face. In the past history it is noted that the patient became jaundiced one month following recovery from the measles. Jaundice as a sequela of measles is uncommon. It would be helpful to know if she had received immune globulin in the treatment of measles. However, the incubation period of serum jaundice is usually from 60 to 120 days. Eight years prior to admission, while in Japan, the patient had beriberi. It is difficult to say what relationship this might have to her present condition.

"The physical examination was not revealing except for the presence of a slight brawny edema of the lower legs and some thickening of the skin over the cheeks, arms and legs. These findings are suggestive of an early scleroderma. There is no mention of atrophy, fibrotic contraction or paroxysmal digital

cyanosis. The laboratory findings during the first hospitalization revealed that the patient had a moderate anemia and leukopenia with a normal differential pattern. Urinalysis showed albuminuria, up to 3 plus, with hyaline casts, pus cells and a quantitative albumin determination of 500 mg. per liter. The significant blood chemical findings include elevated cholesterol and cholesterol esters and a hypoproteinemia with reversal of the albumin-globulin ratio. A urea clearance of 13.5 ml. per minute indicates marked renal impairment. The Congo red test is not helpful in this case. It has been found that in lipid nephrosis a greater quantity of the dye is excreted in the urine than in the normal subject. A retention of only 33 per cent of the dye does not indicate amyloidosis. The laboratory findings are compatible with those found in nephrosis.

"The presence of fluid in the right knee joint and the limitation of motion of the knees and shoulders as noted on her second admission to the hospital indicate a progression of the existing arthralgia. Normal blood pressure readings are again recorded. The absence of fever is somewhat unusual. A febrile course is usually encountered in rheumatic fever or disseminated lupus erythematosus. The recurrence of dermal lesions on the face and extension of similar lesions to the chest during the second hospitalization is of interest. Such cutaneous changes are commonly seen in lupus erythematosus. Evidence of cardiac involvement is manifest at this time by the detection of basal rales and a systolic murmur at the apex. Secondary anemia or pyrexia may be responsible for the murmur but the possibility of verrucous changes in the valves as seen in Libman-Sacks disease must be considered.

"Later in the clinical course the picture is that of severe renal impairment with blood chemical changes indicative of acidosis and uremia. It is noted that the chlorides are reported as 577. If this is meant to be the plasma chlorides it is within the limits of normal; however, this value is elevated for chlorides determined on whole blood. It is usual to find a decreased chloride level accompanying acidosis of renal failure. In view of the progressive renal changes, we must consider periarteritis nodosa as the possible underlying lesion. There is no mention, however, of hypertension, aneurysmal dilatations involving the peripheral vessels or eosinophilia. Shifting dullness in the flanks was found on examination. This could represent ascites or peritonitis following necrotic changes of the abdominal lymph nodes. Two days prior to death the patient had a convulsion which was of short duration. Possible causes include edema of the brain on a uremic basis or vascular changes involving the cerebral vessels.

"In summarizing the findings and clinical course of this patient, we can enumerate the following facts. The patient is a young woman with the initial complaints of arthralgia and an erythematous rash involving the face. Blood counts revealed a leukopenia and anemia. The presence of edema together with the urine and blood chemical findings at first indicated nephrosis which progressed to renal failure. The age and sex of the patient together with the clinical course are entirely in keeping with a diagnosis of disseminated lupus erythematosus."

Dr. Nathan J. Brunner, Department of Dermatology. "We saw this patient during life and considered her disease to be disseminated lupus erythematosus.

"Since the dermatologic manifestations of lupus erythematosus may be unfamiliar to some of those present, a brief general review may be in order. Lupus erythematosus can be divided into two main categories: a purely dermatologic condition (discoid or fixed lupus erythematosus), and a systemic disease with cutaneous manifestations (the acute and subacute disseminated forms). In the discoid form, erythematous plaque-like lesions occur particularly on the face, ears and scalp. On detailed examination of the lesions, telangiectasia and adherent scaling with characteristic "carpet-tack" prolongations extending down into dilated follicles can be distinguished. Older lesions show whitish atrophic stippled scarring, and on the scalp permanent alopecia results. Mucous membrane lesions occur in up to 25 per cent of cases of the discoid variety, appearing as well-defined slightly elevated superficial erosive patches surrounded by radially arranged telangiectatic capillaries. Later, these eroded lesions are covered with a bluish red membrane, and the borders become whitish and thickened. White lines replace the telangiectatic vessels at the margins of the lesions. In the systemic form of the disease, fever, arthritic manifestations, weight loss and symptoms referable to involvement of various viscera become prominent. Some authors consider systemic lupus erythematosus to be a disease which is entirely unrelated to the discoid form. However transitions of one type into the other have been reported, and in fact, in up to 30 per cent of patients with the systemic forms a history of antecedent discoid lupus erythematosus may be obtained. Then too, except for quantitative differences, histopathologic findings in the skin are identical in the two types. Skin manifestations in systemic lupus are variable. In some cases, no skin lesions develop during the entire illness; in others, fleeting or stationary erythematous macules or patches may be seen, particularly on the butterfly area of the face. Diagnosis becomes particularly difficult when, as in the case under discussion, the patient presents no skin changes at the time of examination. Occasionally widespread macular lesions appear, and the "measles" recorded in the resumé of the history may have been an eruption of this type, although the possibility of a toxic erythema from bismuth (which had been employed in treatment by the dermatologist) cannot be ruled out. In hyperacute cases the facial eruption may be vesicular or bullous, and edema may be so great as to simulate severe contact dermatitis. Toxic hair loss is usual, and many patients have cheilitis or painful erosions of the oral and vaginal mucous membranes. With the passage of time, even in acute cases, the development of some follicular dilatation and plugging and telangiectasia may be seen in the skin lesions. In this patient, these characteristic manifestations were first seen in the last few weeks of life.

"As is usual in cases of systemic lupus erythematosus, antibiotics, transfusions and other methods of therapy were of little value. It is of interest that the patient had been subjected to bilateral ovariectomy some years prior to the development of the lupus. Castration and the use of male sex hormone have been recommended in this disease because of its predilection for women in the reproductive period of life."

Dr. Bennett. "Dr. Limarzi, would you comment on the hematologic findings in this and other cases of acute disseminated lupus erythematosus?"

Dr. Louis Limarzi, Department of Medicine. "Most cases of disseminated lupus erythematosus associated with an anemia, leukopenia and at times a thrombocytopenia, present a hypocellular bone marrow. In other cases the bone marrow is normal or hyperplastic. Those cases with a hypercellular marrow usually show a normoblastic reaction secondary to chronic bleeding. Recently, Dr. Hargraves and his associates at the Mayo Clinic (Proc. Staff Meetings, Mayo Clinic, **23**:25, 1948) described a type of cell seen in the bone marrow in cases of acute disseminated lupus erythematosus. These investigators believe these cells are characteristically found in this disease. It is believed that these cells are formed either by phagocytosis of free nuclear material with a resulting round vacuole containing the partially digested and lysed nuclear material, or by actual autolysis of one or more lobes of the nuclei of the involved cell, so that it presents essentially the same appearance as the one which has phagocytized nuclear material. The "L E" cell is practically always a mature *neutrophilic polymorphonuclear leukocyte. These cells, according to* Dr. Hargraves, are found only in the bone marrow in certain cases of acute disseminated lupus erythematosus."

PATHOLOGIC FINDINGS

Dr. G. A. Bennett, Department of Pathology. "Inspection of the body revealed prominent violaceous to brown, scaling lesions over the cheeks, bridge of the nose, left breast, and right and left scapulae. There were also ulcers in the superficial tissues at the mucocutaneous junction of the mouth. The epidermis was thickened over the soles of the feet and there was excessive desquamation. Moderate pitting edema was present over the extremities as well as over the dependent portions of the trunk.

"The *peritoneal cavity* contained 50 cc. of clear yellow liquid and there were moist, filmy adhesions between all organs in the upper half of the abdomen. Mesenteric and periaortic lymph nodes were enlarged. The sections of these nodes indicated marked hyperplasia of the lymphoid follicles and mononuclear cell infiltration in the sinuses. In addition, there were numerous focal areas of necrosis that were accompanied by cellular thrombi in the adjacent sinusoidal channels. No adhesions were present in the pleural or pericardial cavities but serous effusions were noted in the right pleural space and pericardial sac. The right *lung* showed extensive bronchopneumonia in the lower lobe and the hilar lymph nodes were enlarged and softened. The *heart* weighed 250 Gm. Gross and microscopic examinations indicated minimal thickening of the margins of the mitral and tricuspid valves. The stained sections showed minimal amounts of "fibrinoid" change in the subendocardial connective tissues and in the interstitial tissues of the ventricles.

"The *spleen* weighed 190 Gm. There was capsular thickening and the follicles were excessively prominent. On microscopic examination there was pronounced periarterial fibrosis. Occasional vessels showed acute degenerative changes and inflammation of their walls.

"The right *kidney* weighed 180 Gm. and the left, 200 Gm. They appeared moderately pale and swollen. Microscopic examination revealed mild degenera-

tive changes in the tubular epithelium. In the glomeruli, including Bowman's capsule, the basement membrane frequently appeared thickened and a few of the glomerular tufts showed increased cellularity or scarring. Perivascular infiltration with lymphocytes and plasma cells was present in some areas and a few blood vessels exhibited necrosis of their walls.

"On gross inspection the *synovial surfaces* of the right knee and right elbow appeared normal. Microscopically, however, there was minimal evidence of chronic synovitis and a suggestion of "fibrinoid" swelling of the connective tissue in certain portions of the articular capsules.

"Sections of the skeletal *muscles* from numerous areas, including parts near the joints, revealed a pronounced inflammatory reaction in the interstitial tissues about blood vessels. In some regions the walls of small and medium sized arteries were incorporated in the inflammatory change. These lesions were characterized by an intense cellular infiltration with or without degeneration and necrosis of the enclosed connective tissue. Mononuclear leukocytes, lymphocytes, polymorphonuclear neutrophils and eosinophils, comprised the majority of infiltrating cells. Some of the affected blood vessels showed necrotizing changes with superimposed mural thrombi.

"The remaining organs, including the brain and spinal cord, thyroid, one ovary and adrenals, were not remarkable. Specimens from the dermal lesions revealed thinning of the epidermis with lymphocytic and plasma cell infiltration of the subepithelial layers. In some regions the collagen of the corium was fragmented, separated and intensely stained with eosin dye.

"From the anatomic findings it appeared that the manifestations of arthritis were probably attributable for the most part to the widespread involvement of the periarticular tissues rather than to the intra-articular lesions which were minimal."

PRINCIPAL ANATOMIC DIAGNOSES

Disseminated lupus erythematosus with
a. widespread and characteristic dermal lesions
b. serous cavity effusions and adhesions
c. swelling and mild degeneration of connective tissue in heart, skin and blood vessels
d. inflammation and necrosis of blood vessel walls and perivascular tissues in muscle, lymph nodes, spleen and kidney
e. chronic synovitis, mild
f. lymphadenitis

Glomerulonephritis with minimal "wire loop" lesions
Bronchopneumonia

SELECTED ABSTRACTS

The Importance of Accurate Pathologic Classification in the Prognosis of Renal Tumors. N. CHANDLER FOOT, M.D., AND GUSTAVUS A. HUMPHREYS, M.D. Surgery, 23: 369-376, 1948.

The authors reviewed 58 confirmed cases of renal tumors removed by nephrectomy and correlated clinical and pathologic findings with end results. An accurate classification of the renal tumors was thought to be necessary in judging the prognosis. The histogenic, pathologic and clinical findings appeared to justify classifying renal neoplasms into two main subdivisions: those of mesodermal origin and those obviously arising in entodermal conduction apparatus (calices, pelves, ureters). From this the following classification of renal tumors was suggested:

A. Tumors Derived from the Mesodermal Renal Cap
 - Simple adenoma
 - Renal-celled carcinoma, including some tubular forms

B. Tumors Derived from Entodermal Outgrowth from Cloaca
 - Transitional-celled papilloma
 - Transitional-celled carcinoma, epidermoid carcinoma, and some tubular carcinomas (of collecting tubules)

C. Tumors Derived from Embryonal Tissue, such as Mesonephros
 - Embryonal carcinoma (juvenile and adult forms), mixed embryonal tumors, including that of Wilms

D. Tumors Derived from Perirenal Capsule
 - Fatty, connective tissue, muscular and nervous tissue growths

The 58 cases reported were classified as follows: 39 renal-celled, 2 tubular, 5 transitional-celled and 3 epidermoid carcinomas; 4 embryonal tumors (two of them muscular), 2 nonmalignant transitional-celled papillomas, and one Wilms tumor.

The renal-celled carcinomas were characterized histologically by the presence of large vacuolated or clear cells arranged in cords, tubules or, more rarely, papillary formation. The cells occasionally possessed considerable granular cytoplasm and when this was conspicuous, they resembled tubular carcinoma. The authors believe that clear and granular cell elements do not represent separate groups but may be essentially one large family. The transitional-celled tumors were either nonmalignant papillomas or carcinomas arising from the transitional epithelium of the urinary tract derived from the hindgut and hence entodermal. The epidermoid carcinomas were composed of epidermal epithelium thought to have been derived from the transitional cells by metaplasia.

The exact five year postoperative survival rate was determined and proved deaths were also listed for the same period. From these figures the following facts were established:

There was but one five-year survivor in the embryonal group; this tumor was an almost pure leiomyosarcoma discovered incidentally during nephrectomy for tuberculous kidney. With that exception there were no known five-year survivals in that group or among the epidermoid or transitional carcinomas of the kidney, the average expectancy in the latter averaging slightly over one year. In contrast to the transitional-celled and embryonal groups there was a 45 per cent survival in the cases of renal-celled carcinoma. It was also found that there was a definite correlation between the histologic picture and the clinical prognosis in the renal-celled group and that Broder's method of classification was applicable to this group.

From these findings the authors concluded that a classification based upon the embryologic origin of the tumor (entoderm or mesoderm) is reliable in establishing a correlation between the pathologic findings and clinical outcome, with the tumors derived from the mesodermal renal cap offering the best prognosis.

Detroit. OSBORNE A. BRINES

Benign Pelvic Tumors with Ascites and Hydrothorax (Meigs' Syndrome). WILFRED E. WOOLDRIDGE, M.D. and PAUL O. HAGEMANN, M.D. Am. J. Med., **5**: 237–244, 1948.

Meigs and associates brought attention to the syndrome of fibroma of the ovary associated with ascites and hydrothorax. Although Meigs' group has continued to include in the syndrome only fibroma of the ovary, others have broadened the syndrome to include any benign pelvic tumor giving rise to ascites and to hydrothorax. The authors present two cases, both with hydrothorax (right side) and ascites. The ovarian tumor was a fibroma in one case and a pseudomucinous cystadenoma in the other. The pleural fluid in Case 1 changed in character during the preoperative course from that of a simple transudate to a serosanguineous effusion with 65 per cent eosinophils. No explanation is offered for this change.

The most interesting problem relating to this syndrome is the pathogenesis of the pleural effusion. The theories concerning the formation of this effusion are discussed. Several studies have shown that the fluids from the chest and abdomen in the same patient are identical in regard to protein content, specific gravity and electrophoretic studies. The preponderance of evidence seems to indicate that the fluid passes from the abdomen into the chest but the mechanism involved remains problematic.

OSBORNE A. BRINES

Der plötzliche Tod aus natürlicher Ursache bei Soldaten während des vergangenen Krieges. FR. BOEMKE. Frankf. Ztschr. f. Path., **59**: 104–142, 1947.

Boemke reviews sudden natural death in the Nazi armed forces during the late war. A similar study of the United States Army has been made by Moritz and Zamcheck (Arch. Path., **42**: 459, 1946). The first two papers in the most recent series of similar catastrophes in the civilian population have already appeared (Helpern, M., and Rabson, S. M., New York State J. Med., **45**: 1197, 1945; Rabson, S. M., and Helpern, M.: Am. Heart J., **35**: 635, 1948).

The German author judiciously points out that, while extrinsic and intrinsic factors are at work in producing sudden death, one ought never to lay the responsibility exclusively on external features, such as unusual physical exertion. His work, comprising 1816 necropsies, is less effective than it may have been because of the absence of chemical and bacteriologic studies. Extensive investigation may not have been possible during wartime, but, nevertheless, the defect remains. That handicap becomes apparent when Boemke assigns status thymicolymphaticus as a cause of death in 32 of the 1816 instances. He admits, however, that in many of these it is difficult to assay the relative importance of other factors.

In the German series, which includes men over 60 (the American work comprises soldiers up to 40 years of age), 1946 or 81 per cent died of cardiovascular diseases. This, at first glance, is in striking contrast to the 35 per cent of Moritz and Zamcheck. The age distribution of both series, however, serves to dissipate the discrepancy. According to Rabson and Helpern, it is not until the age group 50–54 that cardiovascular diseases provide an undue proportion of victims. It is unfortunate that Boemke does not furnish the age distribution of the Nazi armed forces so that the proper corrections could be made.

Of the 350 American combatants who died of diseases of heart and arteries about 300 (85 per cent) succumbed to coronary arteriosclerosis. That ratio is not far from the 81 per cent of the German statistics. In the American civilian series two-thirds of the cardiovascular group of fatalities were from coronary arteriosclerosis, corresponding to that recorded in the Berlin population several years ago (Hallermann, W.: Der plötzliche Herztod bei Kranzgefässerkrankungen, Stuttgart, 1939, Ferdinand Enke). The higher ratio of coronary arteriosclerotic deaths in the military cardiovascular groups perhaps may be explained by the probable exaggeration in the armed services of the same external factors present in civil life.

Only 70 instances of diseases of the central nervous system are reported by Boemke, whereas 2½ times that number are listed by Moritz and Zamcheck. Comparisons in other disease categories are difficult because the American report is not statistically complete.

Fort Wayne, Indiana S. M. RABSON

Ueber die histopathologischen Veränderungen am Dünndarm bei Störungen der Fettresorption (sog. Xanthomatose des Dünndarms). ROGER FREI. Schweiz. Ztschr. f. Path. u. Bakt., **10**: 685–702, 1947.

Frei, after discussing the current opinions of the digestion and absorption of fat, presents two cases of disturbance of absorption. The first case was that of an 84 year old woman with cerebral arteriosclerotic disturbance who failed to show intestinal dysfunction clinically. In the mucosa and especially in the submucosa of the small intestine there were numerous cells staining red with Sudan III. The other patient was a 43 year old carpenter who suffered with rheumatic joint disease for 4 or 5 years. There was pronounced disturbance of fat resorption, accompanied by marked macrocytic anemia with a lowered color index, and with hypochlorhydria. The sprue-like picture also had serosal inflammation. Large fat droplets were identified in the intestinal walls and in the mesenteric lymph nodes.

Frei considers that the first case of xanthomatosis represents fat storage without tissue damage, and is the early stage of the condition found in Case 2. He adds that "it cannot be clarified whether the basic disturbance rests primarily on an affection of the intestinal epithelium or a chronic intestinal catarrh."

The cases of Frei resemble in some respects those reported recently before the 1948 meeting of the American Association of Pathologists and Bacteriologists (Black-Schaffer, B., Hendrix, J. P., and Handler, P.: Lipodystrophy intestinalis [Whipple's disease]. Am. J. Path., **24**: 677–678, 1948). In the American series the material in the cells of the intestinal wall were regarded as nonlipid, while the mesenteric lymph nodes showed lipogranulomatosis. Black-Schaffer and his co-workers postulated that the condition "is produced by a functional defect of the enteric epithelium resulting in decreased ability to absorb fats, as well as glucose and possibly proteins."

S. M. RABSON

Die Natur der sogenannten Myoblastentumoren. C. WEGELIN. Schweiz. Ztschr. f. Path. u. Bakt., **10**: 631–653, 1947.

Wegelin includes among 10 cases of "Myeloblastentumoren" 2 cases of congenital epulis which, from their gross appearance and location, might be falsely diagnosed. The other 8 cases comprise 1 of the gums, 2 of the tongue, 1 of skeletal muscle and 4 in the skin. In none did the author find any evidence that the "myoblastoma" arises from muscle, and suggests, from both chemical analyses and tinctorial reactions, that not only lipoid, but also protein is stored in the cells. The latter are regarded as of mesenchymal origin, bearing a similarity to the cells in Gaucher's disease. Wegelin, on what appears to be debatable grounds, writes that "the tumors arise in part from nevoid malformations, in part as benign mesenchymal blastomas (lipid-fibroma?) appearing later in life."

Contradictory evidence has been published. Wegelin, in an addendum, cites Feyrter (1946, 1947) and discusses the latter's work in the following sentences: "Probably the myoblast-tumors located in the musculature, expecially those of the tongue, arise from chromotrop-granular altered muscle fibers (but not from myoblasts). As for the remaining sites, however, I must hold fast to derivation from indifferent mesenchymal cells."

The most recent paper was presented at the 1948 meeting of the American Association of Pathologists and Bacteriologists (Fust, J. A., and Custer, R. P.: Granular cell "myoblastomas" and granular cell neurofibromas: separation of neurogenous tumors from myoblastoma group. Am. J. Path., **24**: 674, 1948). Fust and Custer are of the opinion that granular cell tumors of muscle occur only in the tongue and are uncertain whether they are truly neoplastic. Other granular cell tumors are neurogenic and are found chiefly in skin and subcutis, although the tongue may be involved; these are deemed genuine new growths. Fust and Custer separate so-called congenital epulis (also with granular cytoplasm), referred to by Wegelin, and another variety of tumor in skeletal muscle thought to be related to rhabdomyoma from the 51 cases comprising the material of their paper.

S. M. RABSON

Accidental Intra-arterial Injection of Drugs. SOL M. COHEN. Lancet, **2**: 361–371; 409–416, 1948.

Cohen, consultant surgeon to the Gravesend and North Kent Hospital, Gravesend, England, calls attention to accidental injection of soluble thiopentene ("Pentothal Sodium") into the arterial system. Contrary to popular belief, puncture of an artery may require aspiration before blood is seen in the syringe. The crimson tint of oxygenated blood is not readily recognized in the pale yellow anesthetic solution. Not infrequently one may encounter superficial ulnar arteries about the elbow. In fact, in a letter to the editor of The Lancet (**2**: 478, 1948), Simpson remarks that both his own ulnar arteries are superficial, and that it is not an uncommon state judging from his observations in World War I.

The high pH of thiopentene, according to Cohen, is responsible for its pathologic effects. The major vessel is extensively thrombosed, and the distal arterioles are rendered spastic, thus producing sequelae of sloughing cutaneous patches, muscle necrosis, edema of the forearm, nervous disturbance by interference with the blood supply of the main trunks, and gangrene of the limb.

As prophylaxis, it is recommended that vigorous extension of the elbow be avoided, that the injection be limited to "the almost constant vein over the dorsal and lateral aspect of the radius", and that after injection of a small amount of the anesthetic, the anesthetist wait to see if pain appears. Therapy is well described, and the author suggests that "the buffering of thiopentene to render it non-irritating seems within the ken of pharmacologists."

S. M. RABSON

Études sur la "Cellule de Gaucher". SHELMO FRANCO and MOSHE WOLMAN. Schweiz. Ztschr. f. Path. u. Bakt., **10**: 621–630, 1947.

From the Hebrew University of Jerusalem, Franco and Wolman present the first of a series of papers on "staining in the tissues of kerasin, specific cerebroside of kerasinic histiocytosis ('Gaucher's disease')". The anatomic material consisted of 8 human spleens preserved in 10 per cent formalin for a period varying from three months to ten years. After a resumé of current knowledge of kerasin and its presence in reticuloendothelial cells, the authors review attempts to stain the cerebroside. The most recent method (Kahn, J., and Kantrowitz, A. R.: Am. J. Path., **22**: 653, 1946) reveals the substance in crystalline form in the tissues, but does not identify its location in the cell.

Franco and Wolman tried the common lipid stains: Sudan III (Daddi), scarlet red (Herxheimer), Nile blue (Smith), Lorrain-Smith-Dietrich, osmic textroxyde (Mallory), copper acetate-hematoxylin (Fischler), Ciaccio I, and Ciaccio II. The first two methods were most successful, the last two less effective, while the others were useless.

After essaying various steps, the authors developed the following staining method which, they maintain, is specific for kerasin, and does not color other cerebrosides, as, for example, sphingomyelin.

1. Fix in 10 per cent formalin
2. Prepare frozen section
3. Defat (neutral fats)
 a. ether, 15 minutes
 b. acetone, 15 minutes
4. Immerse in boiling water (pH 4), 30 seconds. (Buffering according to Stitt, E. R., Clough, P. W., and Branham, S. E.: Practical Bacteriology, Hematology, and Parasitology. Ed. 10. Philadelphia: The Blakiston Company, p. 925, 1948.)
5. Stain in Sudan III (Daddi), 30 minutes. (Daddi's solution is a warm saturated solution of Sudan III in 80 per cent alcohol)
6. Rinse rapidly in 50 per cent alcohol
7. Pass through distilled water (pH 4?)
8. Stain nuclei with Meyer's hematoxylin

9. Mount in
 a. glycerin-gelatin or
 b. levulose syrup.

S. M. RABSON

Thrombosis as a Factor in the Pathogenesis of Aortic Atherosclerosis. J. B. DUGUID. J. Path. and Bact., **60**: 57–61, 1948.

In an earlier report (J. Path. and Bact., **58**: 207, 1946), Duguid stated that coronary arterial thrombi become organized, forming foci of fibrous thickening indistinguishable from atherosclerosis. The present report presents a similar theme in the aorta, and was made on frozen sections to prevent, among other things, confusion between the identification of fibrin and that of connective tissue. Aortic thrombosis includes not only the obvious status on atherosclerotic ulcers, but a number of microscopic fibrinous incrustations occurring with considerable frequency, even in the young. The end result is intimal thickening after organization.

The larger thrombi at first have condensation of fibrin producing a connective tissue-like appearance. This is succeeded much later by organization, and sometimes by fatty change and even softening. Older fibrin deposits take a variable yellow color with van Gieson's stain, thus suggesting hyalinized fibrous tissue.

The finer, more common form is most often found associated with the larger thrombi, but has been recognized in the otherwise healthy aortas of young children. Under such circumstances they are usually near the ostia of branches where there is superficial fatty streaking. The finer fibrinous incrustations, which may sometimes be mistaken for postmortem clots, pass through the same processes of covering by endothelium, condensation, and hyaline change as the larger lesions.

To check the frequency of the condition, 50 aortas from an unselected series of autopsies were examined with a hand lens. Suspicious areas were then microscopically sectioned. Of the 50 aortas, 19 were affected, of which 15 had the fine type. Atherosclerotic aortas of old persons, of course, had the greatest number, and the "healthy" aortas of children, the least.

Duguid suggests that aortic fatty changes may be both a cause and an effect of thrombosis, reciprocating factors in a vicious circle. As a result, there is thickening of the vessel wall which, in the course of a lifetime, may become severe. Intimal sclerosis, he thinks, depends not only on factors acting on the vascular mural tissues but also on those governing fibrin formation in the circulatory blood.

S. M. RABSON

An Analysis of Certain Factors Associated with the Production of Experimental Dissection of the Aortic Media, in Relation to the Pathogenesis of Dissecting Aneurysm. J. S. ROBERTSON AND K. V. SMITH. J. Path and Bact., **60**: 43–49, 1948.

In an earlier report (Halliday, J. H., and Robertson, J. S.: Proc. Roy. Australian Coll. Physicians, **1**: 3, 1946) it was noted that pressures about 600 mm. Hg were required to produce artificial dissection of the aortic media of the cadaver. The work was continued in an additional 42 cases. From each refrigerated body, 3 pieces (each 2 cm. square) were removed from each of 3 sites: ascending (intrapericardial) portion, arch, and descending (abdominal) portion. A needle, connected with a syringe of water and with a mercury manometer, was inserted parallel to the intimal surface into media approximately in the center of the specimen. Pressure was increased until the endpoint was reached at which a bleb of water formed at the needle point, sent out pseudopods, and then spread through the media.

The pressure required to achieve dissection of the normal media was far greater than that in severest hypertension, even through a medial defect. The authors demonstrated a definite linear gradient of decreasing resistance to dissection; the strongest portion was the ascending segment and the weakest, the abdominal part. There was a weak negative

correlation between dissection-resistance and the age of the subject. There were no sex differences, neither were the degree of associated atheroma and state of the left ventricle correlated with medial resistance, nor was there any association between medial strength and the number of hours between death and the test.

The study prompts several thoughts. The frequency of the rupture of aortic arteriosclerotic aneurysms in the abdominal area as compared to its paucity in the chest is explained not by a greater degree of atheromatosis in the former region, but by the naturally lower medial resistance. Conversely, in the thorax, medial deficiency (in idiopathic cystic medial necrosis) is the chief factor in aortic rupture.

S. M. RABSON

Ueber destruierende, entzündliche Gehirnerkrankungen im frühen Kindesalter. HERBERT FISCHER. Frankf. Ztschr. f. Path., **59:** 247, 1947.

Fischer points out that destructive processes in the brains of children are chiefly identified in their end stage in a category of "porencephalies and scleroses". The porencephalies, despite variation in distribution and localization, are basically smooth-walled, and are devoid of severe connective tissue reaction and evidence of pristine tissue disintegration. Spatz (Ztschr. f. Neurol., **53:** 1919; **167:** 1939; Handbuch der Geisteskrankheiten, **11:** 1930) experimentally demonstrated that immature nervous tissues were destroyed more rapidly than the mature and that the dead tissues were so completely removed, despite slight mesodermal reaction, that smooth-walled glial cysts were produced. The youthful brain (Siegmund: Virchows Arch. f. path. Anat., **241:** 1922) responds to less severe damage with extensive sclerosis which can always be found in the neighborhood of the porencephaly.

In individual cases, however, it may be difficult to rule out birth traumata as responsible for the cerebral alteration. Hence, the case reported by Fischer is of especial interest. The white boy was almost 10 months old when he was admitted to the hospital after a month of "whooping cough" for which vaccines were given. For a day there was low-grade fever and repeated convulsions. The baby lived for $3\frac{1}{2}$ months and the clinical diagnosis during life and at death was post-pertussal progressive encephalitis. At necropsy there was an extensive older meningitis over the entire brain, as well as a circumscribed subdural purulent focus over the right cerebral hemisphere. Especially striking were inflammatory vascular changes irregularly distributed in both hemispheres, and associated with sclerosis and porencephalic softening in various stages of severity. The porencephaly on macroscopic examination at first had been considered as having been caused by birth trauma.

Fischer suggested that from the subdural abscess, perhaps following otitis media, a local "migration" meningitis developed, which later spread over the entire brain. The meningitis led to the vascular alterations with the resultant extensive circulatory disturbances. He rejected edema, produced by inflammation with secondary necrotizing action, as the cause of the final state.

S. M. RABSON

Post-traumatische Osteosklerose der Patella nach dem Typus der Ostitis Deformans Paget. HANS HUNZIKER. Schweiz. Ztschr. f. Path. u. Bakt., **10:** 654–666, 1947.

Hunziker describes the case of a 66 year old man who suffered trauma to his knee 32, 19, and 16 years before death. On the second occasion the patella was fractured, and on the third, the patellar ligament was torn. At necropsy, the kneecap was markedly altered. There was no distinction between cortex and spongiosa, and the basic pattern was one reminiscent of intensely condensed spongiosa. Microscopically, there was the mosaic pattern of bone destruction and formation usually associated with Paget's disease. The photomicrographs, however, are somewhat unsatisfactory so that the reviewer failed to identify all features adequately.

There is very brief reference to the work of Erdheim, in one of which (Ueber die Genese der Paget'schen Knochenerkrankung. Beitr. z. path. Anat. u. z. allg. Path., **96:** 1–60, 1935)

the latter also reminded his readers that a mosaic pattern alone is not pathognomonic of Paget's disease and is seen under many circumstances (rickets, osteomalacia, callus, primary and secondary bone tumors and varied types of hyperostosis). He further emphasized that the mosaic peculiar to Paget's disease rests on definite microscopic features. Bone destruction either does not produce, or at most incompletely produces, a complete resorption-space, and bone formation either does not produce, or at most incompletely produces, a complete haversian lamellar system. Bone destruction and deposition take place in small areas. As a result "the small building blocks in Paget's are not remnants of formerly complete lamellar systems which do not exist here, 'but are originally only fragments of the systems' [Freund], in the same way as the sculptor models by depositing small masses of clay."

Hunzicker's report has its chief value in recalling that there are mosaic patterns in many disturbances of bone, a fact which may be overlooked by the general pathologist who examines but few specimens of bone.

S. M. RABSON

Differential Agglutination of Normal and Sensitized Sheep Erythrocytes by Sera of Patients with Rheumatoid Arthritis. ROSE, H. M., RAGAN, C., PEARCE, E., AND LIPMAN, M. O. Proc. Soc. Exper. Biol. and Med., **68:** 1–6, 1948.

Serial dilutions of sheep's red cells sensitized by rabbit anti-sheep serum and human serum from patients with active rheumatoid arthritis showed agglutination of the erythrocyte in all cases in titers greater than 16. Patients in the inactive stage of the disease were always in the normal range (up to 16). Children with active Still's disease (juvenile rheumatoid arthritis) fell into the same group as the active adult variety. The titer was below 16 in the Marie-Strümpell type of arthritis, rheumatic fever, infectious mononucleosis, arthritis other than rheumatoid or rheumatic fever, and a variety of conditions unassociated with arthritis. It was further found that the agglutinating titer above 16 was unrelated to the sedimentation rate and to the level of antibodies in the serum which will agglutinate Group A hemolytic streptococci. Electrophoretic studies indicated that this substance is found in the globulin fraction of the serum. This test appears to be of practical clinical value in the differential diagnosis of arthritic conditions and in determining the activity of rheumatoid arthritis.

Brooklyn, New York LEO M. MEYER, M.D.

Symposium: Amino Acid Excretion. Transactions of the New York Academy of Sciences, Ser. 11, **10:** 271, 1948.

This symposium consists of four excellent papers by persons actively engaged in the study of amino acid metabolism. They are: Renal Clearances of Essential Amino Acids, by L. D. Wright, The Utilization of Amino Acid Mixtures by Dogs, by Silber, Howe, and Porter, Urinary Excretion of Individual Amino Acids on Normal and Low Protein Diets, by S. W. Hier, and The Urinary Excretion of Amino Acids in Man, by Eckhardt, Cooper, Faloon and Davidson.

Since intravenous nitrogen alimentation is now a commonplace practice the last paper of this group should be of interest to the clinician. Eckhardt and associates discuss the renal loss of the 10 "essential" amino acids following both their intravenous and oral administration. The renal loss of amino acids is greater following intravenous infusion than after oral administration, but only a small proportion of the given amino acids were spilt into the urine. Also, it was found that the excretion of amino acids following parenteral infusion was essentially the same as normal in subjects having either cirrhosis or Wilson's disease.

For those interested in almost any phase of nitrogen alimentation, this symposium should prove to be a valuable compilation of existing data on the wastage of amino acids through renal excretion.

Eloise, Michigan STANLEY LEVEY

Coagulation Defect Produced by Nitrogen Mustard. T. R. SMITH. Science, **107**: 474, 1948.

Five patients with neoplastic diseases were treated with one of the nitrogen mustards, methyl-bis (b-chloroethyl) amine hydrochloride. These patients developed coagulation defects similar to that induced by radiation, which have been previously described by Allen *et al.* under the name "hyperheparinemia". All five patients developed moderate anemia, severe leukopenia, thrombocytopenia, prolonged bleeding time, cutaneous petechiae and ecchymoses. Gastrointestinal and cerebral hemorrhages, resulting in death, were observed in two of the subjects.

Prothrombin times were all normal, and the *in vitro* coagulation time (Lee-White method) was definitely prolonged. In many instances the blood was incoagulable for more than twenty-four hours. When toluidine blue or protamine were administered intravenously (in doses of 2 mg. per Kg. of body weight), the coagulation time was returned to normal for twenty-four hours, *in vivo* and *in vitro*.

Cleveland BEN FISHER

Arteriolosclerosis in a Nine Year Old Child. J. L. GEDGOUD AND J. P. TOLLMAN. Lancet, N. S., **68**: 197–198, 1948.

The authors report a case of moderately severe arteriolosclerosis which occurred in a nine year old girl. The child complained of marked respiratory distress. The blood pressure was 218/176. Urinalyses showed three-plus albumin, specific gravity ranging from 1.015 to 1.028, and white blood cells but no red blood cells. The child continued to be orthopneic and died in cardiac failure. At necropsy, the arterioles in the kidneys, pectoral muscles and spleen showed marked thickening of their walls.

The authors considered that the etiology of the hypertension in this case was possibly associated with a previous pyelonephritis.

BEN FISHER

Experience with Antiheparin Compounds in Essential Thrombocytopenic Purpura. T. W. PARKIN, B. E. HALL and CHARLES H. WATKINS. Proc. Staff Meet., Mayo Clinic, **23**: 309–316, 1948.

The authors review the physiologicochemical action of antiheparin compounds, especially the protamines and toluidine blue. The protamines may have an anticoagulant action, as well as antagonizing heparin. Protamine titrations of normal serums and serums suspected of being "hyperheparinemic" are discussed and the procedure for the titrations is given.

Since previous reports in the literature have suggested that increased amounts of heparin-like substance are present in the blood of patients with primary or secondary types of thrombocytopenia, this study also included clinical observations on a patient with essential thrombocytopenic purpura. Intravenous therapy with protamine sulfate and toluidine blue failed to prevent hemorrhagic phenomena. No conclusive evidence regarding "hyperheparinemia" could be made from protamine-heparin titrations in this case.

BEN FISHER

Relation of Complement to Blood Coagulation. FRANK D. MANN AND MARGARET HURN. Proc. Soc. Exper. Biol. and Med., **67**: 83–85, 1948.

The participation of complement in the blood coagulation process has been disputed for some time. Some workers have found that certain substances have both anticoagulant and anticomplementary properties.

Human plasma, freed of complement by aging and treatment with zymin or ammonia, was studied for the effect of complement in converting prothrombin to thrombin. In the complement-inactive plasmas studied, marked loss of thrombin-forming ability was observed by both the one-stage and the two-stage prothrombin methods. The conversion of prothrombin to thrombin was blocked, but ample reactive prothrombin was left.

BEN FISHER

Use of Radioactive Diiodofluorescein in the Diagnosis and Localization of Brain Tumors. G. E. MOORE. Science, **107**: 569–571, 1948.

Earlier work of the author demonstrated a special affinity of brain tumors for fluorescein. In the present study radioactive iodine (I^{131}) was incorporated into the dye, and after intravenous injection of this compound its relative concentration in various parts of the brain was determined by means of application of Geiger-Müller counters to different areas of the skulls of patients. The differential readings confirmed the increased dye concentration in brain tumors. Correct diagnoses of the presence, or absence, of brain tumors, and of their localization, were obtained in 12 patients, as corroborated by surgery or clinical course.

Chicago KURT STERN

Electrophoretic Studies on the Plasma Proteins of Patients with Neoplastic Disease. I. Gastric Cancer. M. L. PETERMANN and K. R. HOGNESS. Cancer, **1**: 100–103, 1948. *II. An Acid Protein Present in the Plasma.* M. L. PETERMANN and K. R. HOGNESS. Cancer, **1**: 104–108, 1948. *III. Lymphomas and Leukemia.* M. L. PETERMANN, D. A. KARNOFSKY and K. R. HOGNESS. Cancer, **1**: 109–119, 1948.

The first of this series of three papers deals with the electrophoretic pattern of plasma proteins in 25 patients with gastric cancer. The changes observed were decrease of albumin and slight increase of α-globulin and fibrinogen and agreement with the changes found generally in wasting disease. If, however, the electrophoretic studies were carried out at a pH of 4.0, instead of the routinely used pH of 7.6, plasma of patients with gastric cancer was found to contain higher concentrations of an acid protein component than those present in normal persons or in patients with diseases other than cancer. Elevated values of the acid protein were also obtained in cancer of the lung, lymphatic leukemia and Hodgkin's disease.

In the third paper, an analysis of the findings in plasma of patients with Hodgkin's disease, chronic and acute myelogenous leukemia, lymphatic leukemia and lymphosarcoma is presented. The deviations from the normal electrophoretic pattern, noted in these diseases, do not permit a correlation with a specific disease; furthermore, normal patterns were also observed in spite of well-established disease. However, certain changes appeared to occur more frequently in some diseases, such as a rise in α-globulins in Hodgkin's disease.

KURT STERN

Attempts to Produce Cancer in Rhesus Monkeys with Carcinogenic Hydrocarbons and Estrogens. C. A. PFEIFFER and E. ALLEN. Cancer Research, **8**: 97–127, 1948.

The hydrocarbons methycholanthrene, benzpyrene and 1,2,5,6-dibenzanthracene, and estrogens were tested for their possible carcinogenic effect in 50 rhesus monkeys. The experiments were started in 1936, at which time the age of the animals ranged from 3 to 10 years. At the time of this report (1947), only 4 monkeys survived, 3 of which had been treated from 8 to 10 years. The carcinogens were applied by subcutaneous and intravenous injections, cutaneous painting, oral ingestion, implantation in paraffin pellets, or by implantation of crystals in omental pockets. Organs and tissues that are prone to spontaneous tumor development in man (skin, uterus, breast) were exposed to the hydrocarbons in many experiments, and prolonged treatment was employed in most animals.

In none of the animals were any neoplastic lesions produced in this manner. The administration of the hydrocarbons or of estrogens led to local inflammatory reactions, sometimes of granulomatous nature. Marked proliferation of fibrous tissue was often noted, particularly following infection and ulceration of the lesions. Papillary downgrowth of surface epithelium (following painting of skin) and metaplasia of the epithelial lining of the mammary ducts and of cervical glands (following topical application) were encountered. However, these changes never reached true neoplastic character, and they gradually returned to normal after local treatment was discontinued. The gross and microscopic findings are illustrated by 51 photographs.

KURT STERN

BOOK REVIEWS

Occupational Marks and Other Physical Signs. A Guide to Personal Identification. By FRANCESCO RONCHESE, M.D., Instructor in Dermatology, Boston University School of Medicine; Dermatologist in Chief, Rhode Island Hospital, Providence, R. I. 181 pp., 151 figs. $5.50. New York: Grune and Stratton, Inc., 1948.

"Through a number of years of fairly diversified medical experience I have found the study of acquired physical marks and signs for identification purposes and the accumulation of the photographs which illustrate them, an interesting sideline in the routine practice of medicine. Occupational marks or marks due to habits, diseases, accidents, and operations, taken as a group and when carefully evaluated, are of considerable scientific value as an aid to personal identification." These sentences from the author's summary constitute a competent characterization of the scope of the book.

The first chapter entitled, General Aspects, describes the skin as the facade of the human body and touches on fingerprinting, odor and color of the skin, age changes, tattoos, posture, gait, gestures and the general features of occupational marks. The second chapter deals with a wide variety of specific occupational stigmata.

Professional Markings are described in chapter 3. Chapter 4 is concerned with Body Areas and Distinctive Markings, chapter 5 is titled Deceptive Markings and Legal Problems, and chapter 6 is devoted to a Classification of Cutaneous Markings.

The text of this book occupies 56 pages, the illustrations 108 pages and the bibliography 12 pages. The work is well written and the illustrations are excellent. The book should be of especial usefulness to dermatologists, pathologists and medicolegists.

Boston ALAN R. MORITZ

Diagnostic Procedures for Virus and Rickettsial Diseases. Ed. 1. 347 pp. $4.00. New York: American Public Health Association, 1948.

According to the preface written by Dr. Thomas Francis, Jr., this book constitutes an acknowledgment of the increasing demand for a collection of laboratory methods applicable to the diagnosis of virus and rickettsial diseases of man. No pretense was made to an exhaustive review of all possible technics nor any attempt at standardization to the satisfaction of all the authors, Rather, this volume includes the methods found generally workable by those now actively engaged in the field. Such physicochemical procedures as ultracentrifugation and filtration are not specifically discussed and the use of the chick embryo, tissue cultures and neutralization and complement-fixation tests are not taken up in special chapters but are incorporated in the chapters of the different diseases. This has naturally resulted in some repetition with modifications and variations in emphasis by the different authors according to their individual experience. The book is conceived as a manual for the laboratory worker and student and not as a handbook of clinical diagnosis or of theoretical virology. It is a trial flight of limited nature which in future revisions will undoubtedly expand and improve as experience indicates.

Even so, the book is certain to become a "must" in every laboratory. The array of authors includes outstanding workers and authorities on the subjects covered. They are: K. F. Meyer and B. Eddie on Psittacosis; Geoffrey Rake on Lymphogranuloma Venereum; Phillips Thygeson on Trachoma and Inclusion Blenorrhea; Robert F. Parker on Variola and Vaccinia; George K. Hirst on Influenza; A. E. Feller on Primary Atypical Pneumonia; John F. Enders and Jeanette H. Levens on Mumps; John R. Paul on Poliomyelitis; William McD. Hammon on The Encephalitides and Lymphocytic Choriomeningitis; H. N. Johnson and Thomas F. Sellers on Rabies; T. F. McNair Scott on Herpes Simplex; John C. Bugher on Yellow Fever; Albert B. Sabin on Dengue and Sandfly Fever; and Norman H. Topping and Joseph E. Smadel on Rickettsial Diseases.

The material on each subject is presented concisely with adequate paragraph and section

headings which also appear as an outline at the beginning of each chapter and make for easy reading and reference. A detailed general index is also included at the end of the book. The precautions necessary for protection of laboratory personnel and prevention of cross-infection are given in detail as part of each chapter. The presentations of the different authors vary considerably, but several of the chapters contain good tabular summaries which should be useful for differential diagnosis. Others give sample protocols which should prove helpful in the recording and interpretation of the laboratory results. There are also some good illustrations. All in all, this book should prove indispensable even in its present form, and, if its high quality is maintained in future revisions, it will become a bible for laboratory workers in virus and rickettsial diseases.

Boston MAXWELL FINLAND

Pathology. Edited by W. A. D. ANDERSON, M.A., M.D., Professor of Pathology and Bacteriology, Marquette University School of Medicine, Milwaukee, Wisconsin. 1453 pp., 1183 illust., 10 color plates. $15.00. St. Louis: The C. V. Mosby Company, 1948.

In writing a modern textbook on a subject so large and diversified as human pathology, no one author can hope to cover the field adequately without considerable assistance. In the present textbook on *Pathology*, Anderson has had the assistance of 31 collaborators, all of whom have special qualifications for their contributions. The list includes such well known names as Ash, Baker, Bennett, Cannon, Cowdry, Klemperer, Macklin, Moon, Moritz, Pinkerton, Schiller and Warren. While the compilation of a book from the offerings of a variety of authors may result in a lack of uniformity in presentation and may give emphasis on certain diseases out of proportion to their relative importance or frequency, yet the advantages in having authoritative writings and opinions on particular subjects are obvious. It is of interest to note that more than the usual amount of emphasis has been given to discussions on physical and chemical injury, radiation, rickettsial and viral diseases, parasitic and tropical diseases, and vitamin and deficiency diseases. The subject matter and order of presentation, otherwise, are conventional. For the most part, the various topics have been concisely and well covered. There is noticeably little extraneous material. The reader may be somewhat irritated at the excessive use of small print. It is apparent that this must have represented a compromise on the part of the editor. To have used ordinary type without sacrifice of desired material would have made the book too bulky. The illustrations vary considerably in quality: while many of them are excellent and most of them are good, a fair number are poor. The paragraphing is good and the index quite adequate.

Altogether this book will be found to fulfill its intended purposes, *viz.*, to serve as a textbook and as a reference for the practicing and graduate physician. On many subjects it will also serve as a preliminary reference for the pathologist. The selective bibliography should prove to be of value.

Clinical Laboratory Methods and Diagnosis. Ed. 4. By R. B. H. GRADWOHL, M.D., D. Sc., Director of the Gradwohl Laboratories and Gradwohl School of Laboratory Technique; Pathologist to the Christian Hospital. Three Volumes; Volume 1, 1295 pp., 362 figs.; Volume 2, 989 pp., 329 figs., plus index of 127 pp.; Volume 3, 864 pp., 420 figs. $40.00. St. Louis: The C. V. Mosby Company, 1948.

Volume I contains chapters on General Considerations, Urinalysis, Blood Chemistry, Hematology, Blood Groups and Transfusions, Gastric Analysis, Feces, Puncture Fluids and Special Tests. Methods for the examination and a discussion of clinical interpretation are presented under each subdivision. In general, the revision of this portion of the text of previous editions appears to have been largely the insertion of new methods and abstracts of recent literature on interpretation of laboratory results. The bulkiness of the present edition seems to be largely the result of this editorial policy of revision by addition. Nine qualitative tests for albumin in urine and six for sugar are presented without comment. One wonders whether or not the Moore-Heller test for sugar is actually in use today. The difficulties in interpretation of the results of this test were well described by Sahli before

1905. Included in the new material in this volume are sections on phase microscopy; optical and x-ray crystallography, much of which demands physical and optical equipment not available in the average clinical laboratory; physical and mathematical problems of photoelectric colorimetry and modified methods for blood chemical determinations by photoelectric methods; liver function tests; and methods for estimation of enzymes in body fluids.

The chapter on Hematology includes an adequate discussion of the prothrombin time. The chapter on Blood Groups and Transfusions is as nearly up to date as any text book can be. It should be noted that the procedure for the production and standardization of anti-Rh serum of animal origin will not produce a test serum which meets currently accepted criteria. Methods for the establishment and maintenance for a satisfactory blood bank and transfusion service and the safeguards instrumental thereto are well outlined. The chapter on Examinations of Puncture Fluids includes information regarding the use of Turkel instruments for puncture for biopsy. Instruments and technics for bone marrow transfusions are discussed in the chapter on Blood Groups and Transfusions. Medical Mycology occupies only 31 pages of the chapter on Special Tests while the subject of Vitamins and Vitamin Assay is given 52 pages.

Bacteriology, Serology, Postmortem Techniques, Tissue Cutting and Staining, Preparation of Museum Specimens, Toxicology, Crime Detection, Basal Metabolism and Electrocardiology are covered in that order in Volume II. Dr. Felsenfeld, in revising the chapter on bacteriology has adequately presented current bacteriologic knowledge and technics, particularly in the field of enteric bacteriology. One might wish that the bacteriologic methods presented in Volume I had been more adequately correlated with those in the chapter on bacteriology. The reviewer is surprised to learn that Shigella ferment only dextrose. In addition to strictly medical bacteriology, the chapter includes methods in sanitary bacteriology and methods for the diagnosis of rickettsial and virus diseases.

The chapter on Serology includes procedures for a "Wassermann" test and for the performance of the Hecht-Gradwohl complement-fixation test. In the discussion of serodiagnosis of syphilis, no reference was found to the more recent clinical and laboratory work regarding the incidence of false positive reactions nor to quantitative methods for the determination of the significance of positive reactions. Methods for the performance of complement-fixation tests with virus, rickettsial and other antigens are presented.

The chapters on Methods and Technics in Gross and Microscopic Pathology include in addition to standard technics, a new section on surface "biopsy" by the Papanicolaou method with recent modifications.

In the chapter on Toxicologic Technic all of the more common poisons are considered, some of them at great length. Thus, 15 pages are devoted to a discussion of alcohol intoxication and 18 to lead poisoning. The procedures given are largely those employed in the Medical Examiner's offices of New York City and the State of Virginia. Many of the methods given require such highly specialized and expensive equipment that they are hardly suitable for use in the clinical laboratory. This chapter represents a decided improvement over its counterpart in earlier editions.

In the chapter on Detection of Crime by Laboratory Methods, any clinical laboratory which proposes to follow the author's suggestions should provide itself with at least $50,000 worth of spectrographic equipment and obtain the services of a trained physicist. Many of the procedures discussed, such as identification and grouping of blood stains and seminal stains and the microscopic examination of hairs and fibers, can very well be handled in the clinical laboratory. However, discussion of spectrographic methods, identification of fired bullets, use of the polygraph and similar criminologic procedures, appear to be outside the range of the clinical laboratory and might better be left to criminologic investigators.

The material in the chapter on basal metabolism includes discussion of the operation of the 1947 model of the Sanborn machine. The chapter on electrocardiography is new and was written by Julius Elson, M.D.

Volume III is essentially a rewritten text on tropical medicine and parasitology. The material is fully presented. Once again one wishes that the Editor had correlated his

material. Complement-fixation technic in the diagnosis of amebiasis as presented in Volume III duplicates word for word the technic presented in Volume II under Serology.

Summary. Within the 3264 pages of this text one may find methods for the performance of the physical, chemical and bacteriologic tests performed in hospital, clinical, public health and "crime detection" laboratories. In most instances the user will have to exercise his own judgment as to which of the tests presented should be used. In some instances he will have to correlate and evaluate the information scattered throughout the text. That portion of the text classifiable as interpretation of laboratory procedures contains most of the text of the second edition (1938) plus abstracts and excerpts from recent literature.

The bacteriologic portion of Volume II shows more evidence of serious revision, particularly in that portion of the text dealing with enteric bacteriology. Both text and methods have been revised and brought up to date. Correlation of this portion of the text with the presentation of the same subjects in other sections is still indicated. Much of the material presented in the chapters on Toxicology and Crime Detection is not usable in the average clinical or hospital laboratory.

Volume III is essentially a rewritten and excellent text book on tropical medicine.

The book would be more useful had the Editor exercised his scissors more and used his paste pot less.

Lansing, Michigan H. E. COPE
C. W. MUEHLBERGER

The Rhesus Factor. By G. FULTON ROBERTS, M.A., M.B. Second impression. 47 pp., 5 tables. 3s 6d. London: William Heinemann Medical Books Ltd., 1947.

This is a highly condensed presentation of essential information in six brief chapters dealing with the antigen, the antibody, disease due to the rhesus factor, the incomplete antibody, the Fisher nomenclature and treatment. The seventh chapter entitled, The A. B. C. of Specimens, contains valuable instructions on how, when and why specimens should be collected, shipped and examined.

The small pamphlet is full of judiciously selected data, lucidly presented. No faults can be found except for the statement on Page 1 that "agglutination *per se* cannot be observed in the circulation". Actually following transfusion of incompatible blood, small clumps of agglutinated red cells may be seen in preparations made of citrated or oxalated blood.

The discussion of the Fisher nomenclature is particularly well done.

Chicago I. DAVIDSOHN

Microbiology and Pathology. ED. 4. By CHARLES F. CARTER, B.S., M.D., Instructor in Pathology and Applied Microbiology, Parkland Hospital School of Nursing, Dallas, Texas. 845 pp., 216 illus., 25 in color. $5.00. St. Louis: The C. V. Mosby Company, 1948.

This text is designed especially for nurses but should be equally adaptable for technicians and perhaps for some college courses in human biology. The text is revised from the 1944 edition and new chapters on immunization and on the hospital pathologist have been added. Other chapters have been revised in the light of more current work. More than half of the text deals with microbiology and very adequately proves the author's facility in handling this subject. The clinical and laboratory correlation is very well handled. The second major part of the text deals with pathologic anatomy and while the descriptions are very brief, they seem to be adequate. The third part is composed of a series of laboratory exercises which can be used in conjunction with the written text and a final short section consists of a glossary.

At the end of each chapter in the text there is a short review section embracing questions for review, true or false test, completion test and a short but suitable list of references.

This text is particularly strong in clinical pathology and adequate in the other phases covered.

Detroit D. H. KAUMP

Practice of Allergy. Ed. 2. By Warren T. Vaughan, M.D., Richmond, Virginia. Revised by J. Harvey Black, M.D., Dallas Texas. 1132 pp., 333 figs., 81 tables. $15.00. St. Louis: The C. V. Mosby Company, 1948.

Dr. Black has revised the well-known first edition of *Practice of Allergy*, written by the late Warren T. Vaughan in 1939. This revision, started by Dr. Vaughan before his death, has lost none of its facile readability and excellent organization in the hands of Dr. Black.

One of the fine features of the book is the section on the development of our present understanding of clinical allergy, which is presented in a clear and interesting manner. The research of each worker is dated which is helpful to the reader. There is little alteration from the first edition in this section. Other high points in the revised edition are the chapters by Mr. O. C. Durham on aerobiology and field surveys throughout the United States and adjacent areas, by Dr. J. B. Howell on fungous infection with associated allergy, and by Dr. James Holman, an authority on vital capacity. These are new contributions.

The book is extremely well illustrated throughout and, in addition, uses over 1000 references, about 100 (approximately 10 per cent) of which were added during the last three years, 1945 to 1947. The use of reference numbers in the text would have made the book more convenient for research workers using a library. One is somewhat disturbed to find no mention of the theory that antibodies are derived from the lymphocytes in the revised discussion of antibodies. Additional comment on the antihistaminics would not have been out of order.

The over-all excellence of the book far outweighs its few shortcomings. It is a book which should be of particular value for an allergist and for a general practitioner interested in allergy. The difficulties encountered in keeping up with a field of medicine which is changing as rapidly as is allergy make frequent revision of the standard works necessary. Dr. Black has accomplished the job in good style.

Ann Arbor, Michigan John M. Sheldon

Neurosurgical Pathology. By I. Mark Scheinker, M.D., Assistant Professor of Neuropathology and Assistant Professor of Medicine (Neurology), University of Cincinnati, College of Medicine; Neuropathologist and Attending Neurologist, Cincinnati General Hospital. 370 pp., 238 figs. $8.75. Springfield, Ill.: Charles C Thomas, 1948.

This book is intended as a guide to the surgeon who does not have the benefit of a neuropathologic laboratory and must rely on gross pathologic findings and routine histologic methods.

The book is subdivided into five chapters. The first is devoted to the syndrome of cerebral swelling, which is divided into three stages: cerebral tumefaction, cerebral edema, and liquefaction. Disturbances of circulation are regarded as uniformly essential, and the changes in the vessels and in the nervous tissue are described in detail. In this chapter the author resorts, in addition to hematoxylin-eosin staining, to silver impregnations which greatly contribute to the understanding of this important condition. The pathologic picture of herniation of the brain is given consideration. Sixteen critically selected and well reproduced illustrations convey a clear conception of the author's views.

The second chapter deals with injuries of the central nervous system, the classification of which is based upon the "evolutional principle". It includes (1) post-traumatic brain swelling, (2) post-traumatic vasoparalysis, (3) thrombosis, (4) massive hemorrhagic infarction and (5) atropic sclerosis of the white matter. Each of these subdivisions is illustrated by clinical histories and gross and histologic findings. In general, the author succeeds well in outlining the various stages of the injury, beginning with the most acute and ending with that of secondary atrophy.

In the third and largest chapter, the tumors of the nervous system are described. The author follows the generally accepted classification of Bailey and Cushing. A useful addition is that of subependymoma which, according to the author, is derived from the subependymal glia which surrounds the aqueduct, and of infundibuloma of Globus. This chapter is very well illustrated.

In the fourth chapter, abscesses of the brain and of the dura are considered. The acute "malignant" type is distinguished from the chronic, and the subdural abscess is described separately. The author makes extensive use of pictures of gross lesions without, however, neglecting the histology.

In the final chapter there is a short description of hydrocephaly caused by obstruction of the aqueduct by tumors or inflammatory conditions in the aqueduct or meninges.

In general, the author succeeds in outlining the most common and important conditions of interest to a neurosurgeon. The student, the general practitioner, and the young surgeon will find it useful and interesting reading.

Ann Arbor, Michigan K. SCHARENBERG

Le Foie Vasculaire. Angiosclérose Hépatique. By MAURICE FAVRE, Professeur à la Faculte de Médecine de Lyon. 160 pp., 28 figs., 1 plate. Francs 220. Paris: Masson & Cie., 1948.

Favre's small volume prompted this reviewer to examine several American and British textbooks for references to hepatic arterial disease; he could find none. Jores, (in Henke-Lubarsch, Vol. 2, p. 756), discussing aneurysms of the hepatic artery, states that "arteriosclerosis and trauma . . . are to be named as causative factors". The liver changes in malignant nephrosclerosis (Schürmann, R. and MacMahon, H. E.: Virchows Arch. f. path. Anat. **291**: 47, 1933) are described as similar to those in eclampsia, while the arterial alterations are like those seen in the kidney.

Hepatic angiosclerosis is to be found in discrete lesions. The liver should be serially sectioned in slices not more than a centimeter in thickness which can then be read, as Favre remarks, "like the leaves of a book". The best macroscopic picture is obtained after the gross sections are fixed for 24–48 hours in Bouin's solution, for example. Suspicious areas are then cut with a razor or a very fine knife. The sclerotic lesion is "compact, pierced by quite apparent openings." It is white, often with green-stained areas which darken with greater exposure to Bouin's fixative. There is sharp delimitation of the focus from the surrounding hepatic substance.

The lesion is regarded as inflammatory in origin and is limited to the connective tissues. It is located in the "sheaths, the vasculo-biliary pedicles; this term is preferable to that of portobiliary spaces. The sheaths are thickened; it is at their expense that there is formed an elective sclerosis which, involving only some of these pedicles, never has the extension, the generalization which characterizes the cirrhoses (which) always (extend) throughout the entire organ."

Microscopically, the blood vessels are greatly modified, either considerably dilated or contracted and even obliterated. New vessels are formed as the pre-existent channels lose their function. The biliary passages actively proliferate and extend by budding. Nerve elements are numerous and "often attain a development which one is tempted to call monstrous."

In the epithelial portion of the liver, there is vascular and, to a lesser degree, biliary stasis. Necrotic foci may be identified which always start in the sclerotic lesions but, in general, "one can conclude that the integrity of the glandular parenchyma is one of the major characteristics . . . of the vascular liver."

Some of Favre's cases had pulmonary lesions in which the connective tissue "vasculo-bronchial" sheaths were attacked in similar fashion to the "vasculo-biliary" sheaths of the liver. Myocardial sclerotic plaques in the usually enlarged heart are regarded as evidence of former infarcts and as associated phenomena of hepatic angiosclerosis. Recent and older infarcts are considered "the most important and most significant of these renal lesions", unlike the state in malignant nephrosclerosis.

As for etiology, 12 of 60 patients had suffered from syphilis and 5, from tuberculosis. "Nutmeg" liver was found in 11 autopsies. Favre considers that the variety of causative factors is not the important feature, but rather the localization of action of the physiologic-pathologic mechanism which is initiated and which is always the same.

Fort Wayne, Indiana S. M. RABSON.

Standards for the Diagnosis and Treatment of Cancer. By the Cancer Committee of the Iowa State Medical Society. 160 pp. $1.00. Iowa City, Iowa: Athens Press, 1948.

This Manual is a second edition of a compendium for the family doctor who "must help his patients to meet the threat of cancer, when it comes, with open-eyed courage, with determination and equanimity, and with some confidence in the weapons already at hand." It begins with consideration of the incidence and general nature of cancer and the need for, and methods of, lay and professional education now in use. There follow thirty-two sections, each dealing with cancer of a single organ or system under the headings Early Signs, Late Signs, History, Diagnosis, Differential Diagnosis, Treatment and Prognosis. A short section on fundamentals of radiation therapy follows and there is appended a brief bibliography.

It is a concise, "admittedly incomplete and in places didactic" presentation of fundamental facts, up-to-date and conservative in its evaluation of the newer forms of therapy and diagnostic methods. It suggests biopsy and technic for biopsy in those lesions in which such examination is important. There is a surprising amount of well organized information in this small book.

Its first edition, published in 1937, ran to 50,000 copies, and this revision should have wide appeal to the practitioner who wants material at hand to refresh his information without being buried in a mass of detail. The bibliography, classified into sections on cancer of the various organs together with a section on prevention and control, should add to its usefulness.

Kalamazoo, Michigan HAZEL R. PRENTICE

A-B-C's of Sulfonamide and Antibiotic Therapy. By PERRIN H. LONG, M.D., F.R.C.P., Professor of Preventive Medicine, Johns Hopkins University School of Medicine; Physician, The Johns Hopkins Hospital. 231 pp. $3.50. Philadelphia and London: W. B. Saunders Company, 1948.

Although there is little of interest in this manual for the clinical pathologist, it is of inestimable value to the practitioner and student of therapy. The book is a masterpiece of compactness in its handling of a discussion of the pharmacology of the antibiotics and sulfonamides and their clinical application to almost 200 clinical entities. When it is realized that a tremendous amount of clinical study is encompassed in this rather small text, it becomes apparent that Dr. Long has that rare gift in medical writing of getting to the point.

Detroit MARK DALE

Roentgen Studies of the Lungs and Heart. By NILS WESTERMARK, M.D., Director, Department of Radiology, St. Göran's Hospital, Stockholm, Sweden. A series of lectures delivered at the Center for Continuation Study, University of Minnesota. Edited by LEO G. RIGLER, M.D., Professor of Radiology, University of Minnesota. 216 pp., 98 figs. $7.00. Minneapolis: The University of Minnesota Press, 1948. London: Geoffrey Cumberlege, Oxford University Press, 1948.

This book consists of a series of nine lectures given by Westermark at the University of of Minnesota, and include the subjects of emphysema, tuberculosis, lung tumor and embolism. The roentgen findings are based on and correlated with pathologic findings at autopsy, films having been made before and immediately after death. In some cases, casts of the lungs were made. Despite some controversial points, the book has considerable merit and is of value to radiologists, pathologists, and clinicians who are interested in the disease of the heart and lungs.

Detroit A. R. BLOOM

BOOKS RECEIVED

Aureomycin—A New Antibiotic. Annals of the New York Academy of Sciences, Volume 51, Art. 2, pp. 175–342. New York: New York Academy of Sciences, 1948.

OBITUARIES

Louis H. Dyke, Jr.

Louis H, Dyke, Jr. was born in Ecuador, South America, June 29, 1909, and came to this country as an infant. He received his medical degree from the University of Southern California School of Medicine in 1937 and interned at Alameda County Hospital, Oakland, California, 1936–37, remaining there as a resident from 1937 to 1940. He served in the United States Naval Reserve from March 1941 to January 1946, being released as a Commander (M.C.). He was a member of the Board of Directors of the Alameda County Blood Bank; a member of the American Society of Clinical Pathologists since 1941; a diplomate of the American Board of Pathology; a charter member and first President of the recently formed California Pathological Society; and Secretary-Treasurer of the San Francisco Pathological Society. He died February 3, 1948, at his home in Berkeley, California, and is survived by his widow and two children, Margaret Mary, 13, and Louis H., III, 3 years.

James Day Edgar

James Day Edgar was born November 16, 1889 at Redfield, South Dakota. A graduate of Minnesota University, he received the degree of Bachelor of Medicine in 1915. During the First World War he saw military service in Europe. After the Armistice he did notable work in the devastated regions of France, Germany, Russia and Poland. In 1919 he was made Honorary Professor at the University of Krakow, and the Polish government awarded Captain Edgar a medal for work done in the typhus epidemic. In 1921, he became associated with Fitzsimons Hospital, Denver. In the military service, he was assigned to the department of pathology of the University of Oregon Medical School from 1924 to 1932. He then served as pathologist for seven years at the Deaconess Hospital in Spokane, for the next seven years at Mercy Hospital, San Diego, and for the last eighteen months at the Murphy Memorial Hospital in Whittier, California. He is survived by his wife, Bertha Franceska, a resident in Whittier, and by his sister, Mary Wendell Sowdon of Yonkers, New York. Doctor Edgar was a member of the Los Angeles County Medical Association, a diplomate of the American Board of Pathology and a fellow of the College of American Pathologists. He became a member of the American Society of Clinical Pathologists in 1933.

John Stephen Couret

John Stephen Couret, son of the late Dr. Maurice J. Couret, died suddenly April 15, 1948, at the age of 42. He received his medical degree from Tulane University in 1929 and served his residency at Hotel Dieu, New Orleans and at Shreveport Charity Hospital. He served as assistant pathologist under his father at Hotel Dieu through 1946; as Acting director of the pathology department, from January through December 1947; and as director of cardiology, from January 1, 1948, until his death. He was on the faculty of Tulane Medical School as assistant in surgery, 1933–1934, and instructor in pathology and bacteriology, 1934–1939. He became a member of the American Society of Clinical Pathologists in 1940. He is survived by his wife, Mrs. Ethel Wilson Couret; his mother, Mrs. Elda Bougie Couret; and his sister, Mrs. R. Ingram.

Albert M. Harris

Albert M. Harris, pathologist at Sioux Valley Hospital, Sioux Falls, South Dakota, died on January 28, 1948, following an operation for a brain tumor. Dr. Harris was born December 17, 1912, in Nebraska and was graduated from the University of Nebraska in 1939. He was a resident in pathology at University Hospital in Omaha for one and one-half years, and was an instructor in pathology at the University of Arkansas for a similar period.

He then served for three and one-half years as chief of laboratory in an army general hospital in England. He was connected with the Sioux Valley Hospital from October 4, 1946, to his death. He is survived by his widow and four young children. At the time of his death, Dr. Harris was conducting research on a simplified urinary test for cancer utilizing an extract of ground umbilical cord, urine, ether and rabbit serum, which he had reported to be 89 per cent accurate.

August Henry Schade

Doctor Schade was born May 31, 1884 in Saginaw, Michigan and died May 15, 1948. He was graduated from Toledo Medical College and later became a member of the Medical Staff of Robinwood Hospital, Toledo. He joined the National Guard of Ohio in 1910 and served on the Mexican Border until 1914. In 1917, he joined the Medical Reserve Force of the United States Army and was Commanding Officer of the Base Hospital at Montgomery, Alabama until he was sent overseas as a Major in the Medical Corps of the 112th Engineers of Cleveland, Ohio. In 1920, he studied with Dr. George Ives, in St. Louis, taking post-graduate study in Pathology. He became Pathologist for Robinwood Hospital in 1921 and remained in this capacity until the time of his death. He was a charter member of the American Society of Clinical Pathologists and a Counselor of the Society during the years 1933 to 1937, inclusive. A charter member of the Ohio Society of Pathologists organized in 1923, he served as Secretary-Treasurer of this organization until 1946. He was a member of the Academy of Medicine of Toledo and Lucas County, the Ohio State Medical Society and the American Medical Association.

Grant Orante Favorite

Grant Orante Favorite died of heart disease, May 4, 1948, at the age of 44. He was graduated from Hahnemann Medical College and Hospital in 1927. He became an associate in bacteriology and immunology at Jefferson Medical College in Philadelphia and served as professor of bacteriology at his alma mater. During World War II, he was a major in the medical corps of the Army of the United States. He served on the staff of the West Jersey Hospital in Camden, N. J., where the Grant O. Favorite Research Laboratory was established and named in his honor. He was a fellow of the American College of Physicians and a member of the American Society of Clinical Pathologists since 1941.

John George Wurtz

John George Wurtz, of Philadelphia, died January 15, 1948, at the age of 62 of a cerebral hemorrhage. Dr. Wurtz received his medical degree from Hahnemann Medical College and Hospital in 1907, and interned at the Jefferson Medical College in Philadelphia. Dr. Wurtz became a member of the American Society of Clinical Pathologists in 1925. He was certified by the American Board of Pathology and was affiliated with the Women's Homeopathic Hospital, Philadelphia.

AMERICAN BOARD OF PATHOLOGY

General Information

ORGANIZATION

In June, 1935, the Section on Pathology and Physiology of the American Medical Association and the American Society of Clinical Pathologists appointed committees which acted jointly to consider the feasibility and necessity of a national qualifying Board. The joint committees agreed unanimously that such a Board should be established and proceeded to draw up by-laws for the Board. In May, 1936, the American Society of Clinical Pathologists and the Section on Pathology and Physiology of the American Medical Association accepted the proposed by-laws, authorized the nomination of four members each to the Board and suggested incorporation in the state of Michigan. Approval of the Board by the Advisory Board for Medical Specialties and the Council on Medical Education and Hospitals of the A.M.A. was given. On July 19, 1936, the organization of the Board was carried out in Chicago. The original Board of Trustees consisted of Drs. A. H. Sanford, F. H. Lamb, A. G. Foord and R. R. Kracke from the American Society of Clinical Pathologists, and Drs. F. W. Hartman, E. B. Krumbhaar, H. T. Karsner and J. J. Moore from the Section on Pathology and Physiology of the American Medical Association.

Officers elected were: Dr. A. H. Sanford, President; Dr. F. H. Lamb, Vice-President; and Dr. F. W. Hartman, Secretary-Treasurer. These officers served until 1946.

Dr. H. T. Karsner of the A. M. A. Section and Dr. A. G. Foord of the A. S. C. P. whose terms on the Board expired in 1938, were renominated by their respective organizations and were re-elected by the Board.

Dr. E. B. Krumbhaar, who had served as a member of the Board since its inception, resigned in 1940, and his resignation was regretfully accepted. Dr. N. Chandler Foot of New York was elected by the Board to fill Dr. Krumbhaar's unexpired term. Drs. A. H. Sanford and F. H. Lamb were renominated to the Board by the American Society of Clinical Pathologists, and Drs. J. J. Moore and F. W. Hartman were renominated by the Section on Pathlogy and Physiology of the American Medical Association.

At the fall meeting in 1942 two new members, Dr. Paul R. Cannon, Professor of Pathology of the University of Chicago and Dr. Robert A. Moore, Professor of Pathology of Washington University, St. Louis, were elected to the Board under the provision that trustees at large could be elected at the discretion of the Board itself.

In 1943, at its annual business meeting, the American Society of Clinical Pathologists made no nomination for the Board of Pathology in view of the fact that the Section on Pathology and Physiology of the American Medical Association held no meeting and made no nomination. In accordance with the by-laws, Drs. Roy R. Kracke and N. C. Foot remained on the Board until new nominations would be made and elections held.

In 1944, Dr. Roy R. Kracke and Dr. Alvin G. Foord of the American Society of Clinical Pathologists and Dr. H. T. Karsner of the Section on Pathology and Physiology of the American Medical Association had completed two terms and, therefore, under the by-laws, were not eligible for re-election. Dr. James B. McNaught, Professor of Pathology, University of Colorado, and Dr. F. William Sunderman, Director of Laboratories, Cleveland Clinic, were nominated by the American Society of Clinical Pathologists and elected by the Board. Dr. Shields Warren of Harvard Medical School was nominated as the new member by the Section on Pathology and Physiology, and Dr. N. C. Foot was renominated. In addition, the Board elected a third member-at-large, Dr. R. Philip Custer of the Presbyterian Hospital, Philadelphia.

The annual meeting of the Board in 1946 was a most important one. Four members of the Trustees who had served since the founding, Dr. Arthur H. Sanford, Dr. Frederick H. Lamb, Dr. F. W. Hartman and Dr. J. J. Moore, had served two terms. With the election

of their successors, the Board of Trustees was made up entirely of those who had not participated in the initial organization. Dr. James W. Kernohan of the Mayo Foundation and Dr. R. Philip Custer (a member-at-large) were nominated by the A. M. A. Section; and the American Society of Clinical Pathologists nominated Dr. Edwin W. Schultz of Stanford University and Dr. Joseph A. Kasper of Herman Kiefer Hospital, Detroit.

In October, 1948, at the annual business meeting, Dr. Roger D. Baker, Professor of Pathology, Medical College of Alabama, was elected to the Board as Dr. Paul R. Cannon's successor. Dr. Cannon had finished his term and due to the pressure of his other activities, asked to be relieved of his duty to the Board.

Officers of the Board elected in 1946 were re-elected in 1948, as follows: Dr. N. Chandler Foot, President; Dr. F. William Sunderman, Vice-President; and Dr. Robert A. Moore, Secretary-Treasurer.

PURPOSES OF THE BOARD

A. To encourage the study and promote the practice of pathology;
B. To elevate the standards and advance the cause of pathology, by encouraging its study and improving its practice;
C. To determine the competence of those wishing to practice the specialty of pathology and to arrange, conduct, and control investigations and examinations to determine the qualifications of such individuals who voluntarily apply for the certificates issued by the corporation;
D. To grant and issue certificates in the special field of pathology to voluntary applicants therefor and to maintain a registry of holders of such certification;
E. To serve the public, the medical profession, hospitals, and medical schools by preparing and furnishing lists of specialists who have been certified by the corporation.

VALUE OF THE CERTIFICATE

Judging from the experience of other specialties maintaining a certifying board, it is anticipated that the certificate will be of value in that the medical profession, the lay public, and hospital administrators will utilize certificates from the Board as a means of discriminating between those who are thoroughly qualified in pathology and those who are not. Lists of those holding certificates will be made available from time to time through the publication of the same in pamphlets and in national medical journals.

BOARD NOT AN EDUCATIONAL INSTITUTION

The Board is in no sense an educational institution and the certificates of the Board are not to be considered degrees. Therefore, the certificate does not confer on any person legal qualifications, privileges, or license to practice medicine or the specialty of pathology. The Board does not purport in any way to interfere with or limit the professional activities of any licensed physician. Its chief aim, as stated above, is to standardize the qualifications for the specialty of pathology and to issue certificates to those voluntarily complying with the requirements of the Board.

GENERAL REQUIREMENTS

A. General qualifications:

1. Satisfactory moral and ethical standing in the profession;
2. License to practice medicine or a certificate of the National Board of Medical Examiners;
3. Membership or associate membership in the American Medical Association, or membership in a national society acceptable to the Board by citizens of the United States. (At present, these include the American Association of Pathologists and Bacteriologists, the American Society of Clinical Pathologists, the American Medical Associa-

tion, or the American Society for Experimental Pathology.) For citizens of other countries, membership in a national medical society of that country is required;

4. The applicant must devote his time primarily and principally to the practice of pathology.

B. Professional education:

1. Graduation from a medical school in the United States approved by the Council on Medical Education and Hospitals of the American Medical Association, or graduation from a medical school in other countries acceptable to the Board.

C. Special training and experience:

1. The Board admits candidates to examinations who are otherwise eligible and who have had either of the two following types of training and experience:
 a. After five years, if four of the five years have been in institutions approved by the Council on Medical Education and Hospitals of the American Medical Association or by the Board; or
 b. After eleven years, if none of the training and experience has been in institutions so approved.
2. The specific requirements for those acceptable after five years are as follows:
 a. Pathologic anatomy only:
 (1) Four years of supervised study and training in an institution approved for residency training in pathologic anatomy by the Council on Medical Education and Hospitals of the American Medical Association or by the Board. It is immaterial whether the candidate holds the title of resident or fellow or assistant. Candidates may, at their own election, substitute not to exceed 12 months of a straight or rotating clinical internship or a fellowship or instructorship in any of the preclinical departments of a university for 1 of the 4 years. In addition, time, not to exceed 12 months, spent in a department of pathology of an approved school of medicine after the completion of the second year of undergraduate study may be counted for full credit toward the four years.
 (2) One additional year which may be a continuation of the preceding or may be independent practice of pathologic anatomy in a hospital approved by the American Medical Association or other institutions acceptable to the Board.
 b. Clinical pathology only:
 (1) Four years of supervised study and training in an institution approved for residency training in clinical pathology by the Council on Medical Education and Hospitals of the American Medical Association or by the Board. It is immaterial whether the candidate holds the title of resident or fellow or assistant. Candidates may, at their own election, substitute not to exceed 12 months of a straight or rotating clinical internship or a fellowship or instructorship in any of the preclinical departments of a university, for 1 of the 4 years. Candidates, holding also a master's or doctor's degree in a special field of clinical pathology (bacteriology, serology, chemistry, parasitology or hematology) may obtain time credit for not more than 12 to 24 months toward the four years for this work, regardless of whether it was taken before or after the medical degree. The evaluation of time credit will depend on how much of the broad field of clinical pathology was covered in the graduate work.
 (2) One additional year which may be a continuation of the preceding or may be independent practice of clinical pathology in a hospital approved by the American Medical Association or other institutions acceptable to the Board.
 c. Pathologic anatomy and clinical pathology:
 (1) Four years of supervised study and training divided as follows:
 (a) Two years of supervised study and training in pathologic anatomy as outlined in the preceding paragraph C-2-a-(1).

(b) Two years of supervised study and training in clinical pathology as outlined in the preceding paragraph C-2-b-(1). Candidates declared eligible before July 1, 1952, may at their own election substitute one year (12 months) of a straight or rotating clinical internship for one year of this special training in clinical pathology. After July 1, 1952, all candidates applying for both pathologic anatomy and clinical pathology will be required to have two years of supervised training in clinical pathology.

(2) One additional year which may be a continuation of the preceding or may be independent practice of both pathologic anatomy and clinical pathology in a hospital approved by the American Medical Association or other institutions acceptable to the Board. After July 1, 1952, candidates who elect to claim credit of 1 ye r for a clinical internship must take this fifth year as supervised study and training in clinical pathology in institutions approved for residency training in clinical pathology by the Council on Medical Education and Hospitals of the American Medical Association or by the Board. Candidates declared eligible after July 1, 1952, who do not take a clinical internship, may after 4 years of training, as outlined in C-2-c-(1)-(a) and (b), spend the fifth year in independent practice in an institution or laboratory acceptable to the Board.

Note: As outlined in the preceding paragraphs, the total time requirements of the Board are unchanged after July 1, 1952, namely, five years of study or practice after graduation from medical school (with exceptions noted in sections C-2-a-(1) and C-2-b-(1)).

3. The specific requirements for those acceptable after 11 years are as follows:
 a. The practice of pathology under circumstances acceptable to the Board for a period of not less than 11 years. At the election of the candidates, a period not to exceed 1 year of straight or rotating clinical internship may be substituted for 1 of the 11 years. For those candidates in this category who have had some special study and training in pathologic anatomy or clinical pathology acceptable under paragraphs C-2-a, b, or c, double time credit will be allowed. Thus, if a person has 2 years of acceptable supervised study and training, only 7 years of practice are required.

D. Special qualifications:

1. Prior to December 31, 1947, the Board at its discretion certified candidates without examination, if the following conditions were met as of July 1, 1939:
 a. That the candidate had been for a period of 5 years of professorial rank in a department of pathology in an approved medical school, or
 b. That the candidate had been practicing pathology for 10 years in a senior position in a hospital having an adequate department of pathology, approved by the Council on Medical Education and Hospitals of the American Medical Association, or in an institution acceptable to the Board.

CREDIT FOR MILITARY SERVICE

Credit may be allowed for training and experience in pathology in the Federal Services during the period from July 1, 1940 to June 30, 1947. This credit for training or experience or both is given on an individual basis and will depend upon the opportunity the applicant has had, as indicated by his or her medical service record in the specialty of pathology.

After July 1, 1947, credit for those in military services will be given on the same basis as it is in civilian institutions, except that the rule in the preceding paragraph will not apply to reserve officers who continue on active duty or are called to active duty after that date.

SUBSPECIALTIES OF PATHOLOGY

(In Force after July 1, 1948)

Candidates who have met all general requirements and who have successfully passed the examination in pathologic anatomy and clinical pathology may apply for special designation of a subspecialty of pathologic anatomy or clinical pathology. The Board, at its discretion, may approve this application, and after the candidate has successfully passed a prescribed examination, will issue a certificate designating the subspecialty, reading "Pathologic Anatomy (Neuropathology)", "Clinical Pathology (Clinical Bacteriology)", *etc.*

Candidates who have met all general requirements and who have had five years of special training and experience in a special field of pathology which is acceptable to the Board may apply to the Board for certification in that special field. The Board, at its discretion, may approve this application, and after the candidate has successfully passed a prescribed examination, will issue a certificate reading "Neuropathology", "Clinical Bacteriology", *etc.*

APPLICATION BLANK AND FEE

Application must be made on the special form which may be procured from the Secretary and forwarded with other required credentials and the application fee. An application cannot be given consideration by the Board unless it is accompanied by the application fee.

The application or examination fee for candidates is $50.00. If the candidate fails in his examination, he will be admitted to a second examination after one year, but not later than three years, without additional fee. After two re-examinations, the applicant must file a new application and pay an additional fee before a fourth examination will be given.

The application fee of $50.00 has been determined after careful consideration and is based on actual estimates of the expense of examination and administration. None of the Board members receive any compensation for their services except actual expenses incurred.

If the applicant, for any reason, is deemed ineligible for examination by the Board, his fee will be returned; however, the application fee is not returnable after the candidate has officially been accepted, and notified to report for examination.

EXAMINATIONS

Written and oral examinations will be held at the discretion of the Board at or near the time and place of national medical meetings. If a number of applications from any region of the country are received, an examination in conjunction with a national medical meeting in that section will be arranged so that the financial outlay of the applicant in meeting the examiners will be as small as possible.

The examinations are to be based on the broad principles of pathology with emphasis on diagnosis, interpretation, and technic. The applicant may apply for certification in either pathologic anatomy or clinical pathology or both.

The examination in pathologic anatomy consists of a written test, an oral examination on gross pathology and a practical examination in microscopic pathology. The examination in clinical pathology consists of a written test and an oral and practical examination in the six phases of clinical pathology: bacteriology, hematology, clinical chemistry, parasitology, serology and clinical microscopy.

DEFINITIONS

1. Pathologic anatomy is that branch of pathology which deals with the morphologic aspects of disease, recognition being given that that definition covers two phases of pathology:
 a. The applied phase with special attention to the description and diagnosis of gross and microscopic specimens; and
 b. The academic phases of teaching and general morphologic diagnosis.

2. Clinical pathology is that branch of pathology which deals with bacteriology, immunology, clinical chemistry, parasitology, hematology, endocrinology, and clinical microscopy, the application of the physical and biological sciences to the diagnosis, prognosis and treatment of disease.

REVOCATION OF CERTIFICATES

Certificates issued by the American Board of Pathology are subject to the provision of the Articles of Incorporation, No. II, Section 4. Any certificate issued by the Board of Trustees shall be issued subject to revocation in the event that:

1. The physician or party so certified shall have made any misstatement or misrepresentation of a material fact in his application, or in any other communication to the Board or its representatives, which misstatement or misrepresentation affected the eligibility of the physician or party so certified;
2. The issuance of such certificate has been made contrary to or in violation of any of the rules, laws, or regulations of this corporation;
3. It is ascertained that the physician or party so certified is not eligible in fact to receive such certificate;
4. The physician or party receiving such certificate shall, prior to the issuance of such certificate or thereafter, have been convicted by a court of competent jurisdiction of a felony or of any misdemeanor, which misdemeanor in the opinion of the Board of Trustees, shall involve moral turpitude;
5. The physician or party receiving such certificate shall, prior thereto or thereafter, have his license to practice medicine revoked;
6. The physician or party receiving such certificate shall be expelled from any of the societies or organizations that made him eligible to this corporation as an applicant for such certificate.

CRITERIA FOR APPROVAL OF INSTITUTIONS FOR TRAINING IN PATHOLOGY

In Sections C-2-a, C-2-b, and C-2-c of the General Requirements, it is stated that candidates must have certain periods of supervised study and training. The American Board of Pathology, in cooperation with the Council on Medical Education and Hospitals of the American Medical Association, certifies hospitals in the United States as satisfactory for this supervised study and training. Lists of these hospitals are published in the Educational Number of the Journal of the American Medical Association each year. In addition, the American Board of Pathology recognizes certain hospitals outside the United States and certain laboratories not connected with hospitals in the United States. Inquiries concerning these should be directed to the Secretary of the Board.

The general criteria for approval of hospitals and laboratories are both qualitative and quantitative. On the score of quality, consideration is given to the qualifications of the Director of Laboratories and the associates and assistants, the supervision of work of the person in training, the excellence of the educational program, and the exactness and completeness of the laboratory work performed. On the score of quantity, consideration is given to the volume and distribution of laboratory work, both in absolute numbers and in relation to the size of the hospital, to the diversity and completeness of tests performed, to the size and equipment of the laboratory, and to the number of professional and nonprofessional personnel in relation to the volume of work.

In general, the qualitative standards will determine whether or not a hospital or laboratory is approved, and the quantitative standards will determine whether the approval is for 1, 2, 3, or 4 years of credit toward the requirements of the Board.

In evaluation of applications the Board takes into consideration the following criteria:

1. Director of laboratories or pathologist:
 a. It is required that the responsible head of the laboratory hold the certificate of the American Board of Pathology in the subject for which the hospital is approved, or

be eligible for certification, and that he or she spend full-time in the hospital. Full-time is not interpreted in terms of hours, but rather that the director have no obligations outside the one approved hospital except in a university department of pathology where he and the residents have an opportunity to participate in the educational program;

b. In special instances, the equivalent of full-time by two or more qualified individuals will be accepted and one person need not spend the entire working day in the laboratory;

c. In special instances, two or more hospitals will be approved as a unit with a single full-time director of laboratories if it is apparent that a satisfactory training program can be conducted;

d. In hospitals with over 350 beds, it is expected that the professional staff, in addition to the pathologist, will include one or more persons with special training and qualifications in the subspecialties of clinical pathology.

2. Technicians:
There are no absolute criteria, but it is expected that the number of technicians will be proportional to the volume of laboratory work and that, insofar as possible, the technicians will hold the certificate of the Registry of Medical Technologists of the American Society of Clinical Pathologists.

3. Floor space of laboratory:
In general, it is believed that the size of the laboratory should be related to the size of the hospital and the volume of laboratory work. A minimal ratio is 4 square feet of space in the laboratory, including morgue and autopsy room, for each bed in the hospital.

4. Equipment for the laboratory:
The variety and completeness of laboratory tests performed depends on the size of the hospital. In all hospitals there should be facilities for the more common tests in clinical pathology, for study of surgical specimens, and for performance of autopsies. In larger hospitals, the variety of tests performed should be larger and in hospitals with over 500 beds all recognized laboratory procedures should be available for study and treatment of the patients.

5. Autopsy percentage:
No institution with a percentage of less than 15 will be approved, and those institutions with percentages between 15 and 40 will be given special scrutiny.

6. Examination of surgical specimens:
All surgical specimens should be sent to the laboratory for gross examination, and microscopic examinations should be made unless there are general or special reasons not to do so.

7. Indices:
There should be indices according to the names of the patients and the diagnoses of all surgical and autopsy material. Indices of clinical pathology are left to the discretion of the hospital.

8. Museum:
There should be available fixed anatomic and pathologic specimens for study by the staff in proportion to the size of the hospital. Properly filed and indexed color photographs may in part be substituted for museum specimens.

9. Library:
A reasonably complete library of modern books and recent unbound and bound journals should be available to the hospital, and the more commonly used books and journals should be on hand in the laboratory.

10. Educational program:
The work of the person in training should be supervised. Conferences, seminars, journal clubs, and demonstrations should be conducted as frequently as the volume of material and the size of the staff justifies. A clinicopathologic conference must be held at least every 2 weeks.

11. Volume of laboratory work:
 a. As indicated in the following categories, no hospital with less than 75 autopsies, 1000 surgical specimens, and 25,000 tests in clinical pathology annually will be approved (Category D), except as outlined in the following paragraphs. It is the belief of the Board that less material than this is inadequate for the training of a pathologist.
 b. In the field of clinical pathology, there should be a reasonable diversification of tests and in each category there should be sufficient absolute volume to provide training and experience. There are no absolute criteria, but special scrutiny will be given to a hospital in which there is not a reasonable diversification and variety.
 c. In the field of pathologic anatomy, a deficiency in either autopsies or surgical specimens may be made up by an excess of the other, if the deficiency does not exceed 20 per cent of the minimum required in the ratio of 1 autopsy to 75 surgical specimens. Thus in a hospital approved for one year in pathologic anatomy and clinical pathology, the minimums are 75 autopsies and 1000 surgical specimens. If a hospital has 2500 surgical specimens, it is acceptable if there are only 60 autopsies.

If a hospital has met these minimal qualitative and quantitative standards, it will then, on the basis of the following quantitative standards, be approved for 1, 2, 3 or 4 years of training in pathologic anatomy, or clinical pathology, or both, or some special field as shown for the number of residents indicated.

Category A. In both pathologic anatomy and clinical pathology for 4 years (as required of all candidates seeking certification in both fields who are examined after July 1, 1952).

Minimum: 150 autopsies, 1750 surgical specimens, and 65,000 tests in clinical pathology for 4 residents. Additional resident for each 50 autopsies or 500 surgical specimens, or 20,000 tests in clinical pathology.

Category B. In both pathologic anatomy and clinical pathology for 3 years. This meets all requirements of the Board for supervised training until July 1, 1952. Candidates examined after that date who seek certification in both subjects must take an additional year of supervised training in clinical pathology or pathologic anatomy, in another hospital, which is approved for the deficiency of training required.

Minimum: 125 autopsies, 1500 surgical specimens, and 50,000 tests in clinical pathology for 3 residents. Additional resident for each 50 autopsies, 500 surgical specimens, or 20,000 tests in clinical pathology.

Category C. In both pathologic anatomy and clinical pathology for 2 years. Candidates taking two years in these institutions must have an additional year (2 years after July 1, 1952) in an institution in category A, B or D.

Minimum: 100 autopsies, 1250 surgical specimens, and 40,000 tests in clinical pathology for 2 residents. Additional resident for each 50 autopsies, or 500 surgical specimens, or 20,000 tests in clinical pathology.

Category D. In both pathologic anatomy and clinical pathology for 1 year. Candidates taking training in these institutions must have an additional 2 years (3 years after July 1, 1952) in institutions which are approved for the deficiency of training required.

Minimum: 75 autopsies, 1000 surgical specimens and 25,000 tests in clinical pathology for 1 resident. Additional resident for each 50 autopsies, or 500 surgical specimens, or 20,000 tests in clinical pathology.

Category E. In pathologic anatomy only, for 3 or more years. Candidates taking all training in these institutions will not be eligible for certification in clinical pathology unless an additional year (2 years after July 1, 1952) is taken in clinical pathology in institutions which are approved for the deficiency of training required.

Minimum: 175 autopsies and 1500 surgical specimens for 3 residents. Additional resident for each 50 autopsies or 500 surgical specimens.

Category F. In pathologic anatomy only, for 2 years, Candidates taking training in these institutions will not be eligible for certification in clinical pathology unless they take

one additional year full time in clinical pathology (2 years after July 1, 1952) in institutions which are approved for the deficiency of training required, and will be eligible in pathologic anatomy only if another year is taken in another institution which is approved for one or more years in pathologic anatomy.

Minimum: 125 autopsies and 1250 surgical specimens for 2 residents. Additional resident for each 50 autopsies or 500 surgical specimens.

Category G. In pathologic anatomy only, for 1 year. Candidates training in these institutions will not be eligible in both pathologic anatomy and clinical pathology unless they take an additional year in pathologic anatomy and an additional year (2 years after July 1, 1952) in institutions which are approved for these periods of training. Candidates seeking certification in pathologic anatomy only must study an additional 2 years in institutions which are approved for that period of training.

Minimum: 75 autopsies and 1000 surgical specimens for 1 resident. Additional resident for each 50 autopsies or 500 surgical specimens.

Category H. For postmortem part of pathologic anatomy for 2 years. Credit is never allowed for more than 2 years. Candidates taking training in these institutions may pursue further training as follows: 1. For pathologic anatomy only. An additional year in an institution in category A, B, C, E, F or G with assignment to surgical pathology principally. 2. For pathologic anatomy and clinical pathology. An additional year (two years after July 1, 1952) in an institution in category A, B, C, L, M or N with assignment to clinical pathology and ½ year assigned to surgical pathology principally, in an institution in category A, B, C or J.

Minimum: 125 autopsies for 2 residents. An additional resident for each 60 autopsies.

Category I. For postmortem part of pathologic anatomy for 1 year. Candidates taking training in these institutions may pursue further training as follows: 1. For pathologic anatomy only. An additional two years in an institution in category A, B, C, E, F or G with general assignments. 2. For pathologic anatomy and clinical pathology. An additional year (2 years after July 1, 1952) in an institution in category A, B, C, L, M or N with assignment to clinical pathology and 1 year to an institution in category A, B, C, E, F or G.

Minimum: 75 autopsies for 1 resident.

Category J. Surgical pathology part of pathologic anatomy for 1 year. Credit is never allowed for more than 1 year and the director of the laboratory must hold the certificate of the American Board of Pathology in pathologic anatomy or be eligible for certification. Candidates training in these institutions may pursue further training as follows: 1. For pathologic anatomy only. An additional 2 years in an institution in category H or in an institution of category A, B or C with assignment to postmortem pathology only. 2. For pathologic anatomy and clinical pathology. An additional year (2 years after July 1, 1952) in an institution in category A, B, C, L, M or N with assignment to clinical pathology full time, and an additional year to an institution in category A, B, C, D, E, F, G or H with assignment to postmortem pathology full time.

Minimum: 2000 surgical specimens for 1 resident. An additional resident for each 1000 surgical specimens.

Category K. Special pathology as part of pathologic anatomy. Credit for not to exceed ¼ the time credit (1 year if seeking certification in pathologic anatomy only, 6 months if seeking both pathologic anatomy and clinical pathology) of candidates applying in pathologic anatomy may be taken in special laboratories with limited activities such as neuropathology, orthopedic pathology, ophthalmic pathology, etc. The candidate who receives credit in this category may, on request, have the field of special pathology designated on the certificate of the Board.

Category L. Clinical pathology for 3 or more years. Candidates taking 3 years of training in these institutions will not be eligible for pathologic anatomy unless they take an additional 2 years in institutions approved for pathologic anatomy.

Minimum: 100,000 tests in clinical pathology for 3 residents. Additional resident for each 50,000 tests.

Category M. Clinical pathology for 2 years. Candidates taking 2 years' training in these institutions must take the same additional training as in category L to be eligible for pathologic anatomy also. To be eligible for clinical pathology only, an additional year of clinical pathology must be taken in an approved institution.

Minimum: 75,000 tests in clinical pathology for 2 residents. An additional resident for each 50,000 tests.

Category N. Clinical pathology for 1 year. Candidates taking training in those institutions must take an additional 2 years in an institution in category A, B, C, L or M assigned to clinical pathology for eligibility in clinical pathology only. To be eligible in pathologic anatomy and clinical pathology, an additional 2 years in approved institutions is required.

Minimum: 50,000 tests in clinical pathology for 1 resident.

Category O. Special clinical pathology as part of clinical pathology. Candidates applying for clinical pathology only may receive credit for not in excess of $\frac{1}{4}$ of the training period (1 year if seeking certification in clinical pathology only, six months if seeking both pathologic anatomy and clinical pathology) for work in a special field of clinical pathology, such as bacteriology, serology, etc. Under these circumstances, on request, the special field will be designated on the certificate of the Board.

Category P. Research: Residence in certain institutions in which full time is devoted to research with a direct application to the practice of pathologic anatomy or clinical pathology may be accepted for credit not to exceed one-third the time requirement (20 months if no clinical internship, 16 months if a clinical internship was taken). The Board encourages research and believes that all candidates should carry on investigation during their training in all institutions. Therefore, this category is only for those wishing to do full-time research.

ALPHABETIC LISTING OF DIPLOMATES

ABEL, ARTHUR R., Orange, N. J.
ACKERMAN, LAUREN V., St. Louis, Mo.
ACKERMAN, MILTON, Aspinwall, Pa.
ADAMKIEWICZ, L. L., Philadelphia, Pa.
ADAMS, CHARLES F., Jefferson City, Mo.
ADAMS, GEORGE, New Orleans, La.
ADAMS, JOHN W., JR., Chattanooga, Tenn.
ADLER, DAVID LEO, Columbus, Ind.
ADLER, JACOB, New York, N. Y.
AEGERTER, ERNEST E., Philadelphia, Pa.
AHRONHEIM, J. H., Jackson, Mich.
AKERSON, IRVING, Bridgeport, Conn.
ALBANO, EDWIN H., East Orange, N. J.
ALEXANDER, MARSHALL O., Rockford, Ill.
ALEXANDER, W. STEWART, Dunedin, New Zealand
ALLEBACH, HOBART K. B., Kansas City, Mo.
ALLEN, ARTHUR C., New York, N. Y.
ALLEN, HENRY C., St. Louis, Mo.
ALLEN, HOLLIS N., St. Louis, Mo.
ALLEN, J. D., Louisville, Ky.
ALLEN, PLINY, Omaha, Neb.
ALLEN, WILLIAM HOGUE, Louisville, Ky.
ALTER, NICHOLAS, Jersey City, N. J.
AMOLSCH, ARTHUR, Marquette, Mich.
ANDAY, GEORGE J., Chicago, Ill.
ANDERSEN, DOROTHY, New York, N. Y.
ANDERSON, MELVIN W., Alhambra, Calif.
ANDERSON, W. A. D., Milwaukee, Wis.
ANDREWS, VERNON L., Glendale, Calif.
ANDUJAR, JOHN J., Ft. Worth, Tex.
ANGEVINE, D. MURRAY, Madison, Wis.
ANGRIST, ALFRED, Jamaica, N. Y.
ANTOPOL, WILLIAM, Newark, N. J.
APPEL, MAX, Champaign, Ill.
APPERLY, FRANK L., Richmond, Va.
AQUINO, JUAN ARCELLANA Y., Chicago, Ill.
ARBOGAST, J. L., Indianapolis, Ind.
AREY, JAMES BLANDING, New Orleans, La.
ARONOFF, ROSA, New York, N. Y.
ARONSON, JOSEPH, Philadelphia, Pa.
ARONSON, ROLAND, APO 500, % Postmaster, San Francisco, Calif.
ARONSON, WILLIAM, New York, N. Y.
ARONSTEIN, CHARLES G., Washington, D. C.
ASH, JAMES EARLE, Washington, D. C.
ASHBURN, L. L., Bethesda, Md.
ASHWORTH, CHARLES T., Fort Worth, Tex.
AUERBACH, OSCAR, New York, N. Y.
AUERBACH, STEWART H., Nashville, Tenn.
AUSTIN, J. HAROLD, Philadelphia, Pa.
AYER, GUY D., JR., Atlanta, Ga.
AYERS, AMOS J., Atlanta, Ga.

BACH, AMIL C., Kansas City, Mo.
BACHER, WILHELMINA, New Orleans, La.
BACKUS, G. R., Flint, Mich.
BAGGENSTOSS, ARCHIE H., Rochester, Minn.
BAILEY, ORVILLE T., Boston, Mass.
BAILEY, WILLIAM H., Excelsior Springs, Mo.
BAIRD, ELWOOD E., Denver, Colo.
BAISINGER, CECIL F., Riverside, Calif.
BAKER, CHARLES P., Oakland, Calif.
BAKER, MARGARET AUGUSTA, Brooklyn, N. Y.
BAKER, MARGARET R., Akron, Ohio
BAKER, ROGER D., Birmingham, Ala
BAKER, THEODORE, JR., Kingston Pa.
BALDAUF, LEON K., Cambridge, Mass
BALKEN, RUTH B., Gary, Ind.
BALL, HOWARD A., San Diego, Calif.
BANKS, HORACE W., Indianapolis, Ind
BARBROW, A. L., Homestead, Pa.
BARLAND, SAMUEL, Elmhurst, N. Y.
BARNARD, ROBERT D., New York, N. Y.
BARNES, MARIAN, Chicago, Ill.
BARNETT, ROY NATHANIEL, Norwalk, Conn
BARON, EDGAR, Kecoughtan, Va.
BARTA, EDWARD F., Milwaukee, Wis.
BARTLETT, CHARLES J., New Haven, Conn.
BASS, ELIZABETH, New Orleans, La.
BATES, LEWIS B., East Weymouth, Mass.
BAUER, JOHN T., Morristown, N. J.
BAYLESS, FRANCIS, Cleveland Heights, Ohio
BAYLEY, WILLIAM E., Lafayette, Ind.
BAYLISS, MILWARD, Ft. Sam Houston, Tex.
BEAM, MARK P., Albuquerque, N. M.
BEAUREGARD, JOHN F., Pittsburgh, Pa.
BEAVER, DONALD C., Detroit, Mich.
BECK, JAMES S. P., Tuscaloosa, Ala.
BECK, REGENA C., Richmond, Va.
BECKER, CHARLES F., Buffalo, N. Y.
BECKETT, RONALD S., New York, N. Y.
BEECROFT, MORRIS, Newport News, Va.
BEEMAN, JOSEPH, Boise, Idaho
BEISSINGER, HEINZ, Baltimore, Md.
BELDING, DAVID L., Boston, Mass.
BELK, WILLIAM P., Ardmore, Pa.
BELL, ELIXIOUS T., Minneapolis, Minn.
BELL, MARVIN DEWITT, Dallas, Tex.
BENICHEK-LANGFORD, MARIA, Himsdale, Ill.
BENJAMIN, EUSTACE L., San Bernardino, Calif.
BENNER, MIRIAM C., Denver, Colo.
BENNETT, A. P , Evansville, Ind.
BENNETT, GRANVILLE A., Chicago, Ill.
BENNETT, WARREN, Rochester, Minn.
BENNETT, WEBSTER W., Aspinwall, Pa.
BERDEZ, GEORGE L., Duluth, Minn.
BERGER, LOUIS, Quebec, Canada
BERGSTROM, VICTOR W., Binghamton, N. Y.
BERMAN, LAWRENCE, Detroit, Mich.
BERNHARD, WILLIAM G., Short Hills, N. J.
BERNHEIM, ALICE I., New York, N. Y.
BETTIN, MONA E., Los Angeles, Calif.
BEVANS, MARGARET, New York, N. Y.
BEVEN, JOHN L., Baton Rouge, La.
BEVILACQUA, MICHAEL, Jamaica, N. Y.
BINFORD, CHAPMAN, Baltimore, Md.
BIRGE, EDWARD A., Milwaukee, Wis.
BIRGE, R. F., Des Moines, Iowa
BIRON, ROBERT E., Manchester, N. H.
BISHOP, EVERETT L., Atlanta, Ga.
BISKIND, GERSON R., San Francisco, Calif.
BITAR, EMMANUEL, Everett, Wash.
BLACK, SAM H., Louisville, Ky.
BLACK, WILLIAM C., Denver, Colo.
BLACK-SCHAFFER, BERNARD, Durham, N. C.
BLEYER, LEO F , Madison, Wis.
BLOCK, NATHAN, New York, N. Y.
BLUM, LEON L., Terre Haute, Ind.

BLUMBERG, ALFRED, Atlanta, Ga.
BLUMBERG, JOE M., Augusta, Ga.
BLUMENFELD, CHARLES, Sacramento, Calif.
BLUMENTHAL, HERMAN T., St. Louis, Mo.
BLUMSTEIN, CHARLES L., Columbus, Ga.
BOCIAN, J. J., Fresno, Calif.
BOGEN, EMIL, Olive View, Calif.
BOHLS, SIDNEY W., Austin, Tex.
BOHROD, MILTON G., Rochester, N. Y.
BOLAND, JOSEPH, Albany, N. Y.
BOLIN, SERA E., San Francisco, Calif.
BOLKER, HERMAN, Brooklyn, N. Y.
BONYNGE, CHARLES W., Los Angeles, Calif.
BORNSTEIN, FREDERICK, Herrin, Ill.
BORNSTEIN, SIEGBERT, Brooklyn, N. Y.
BOSSE, MILTON, Pitcairn, Pa.
BOSTICK, WARREN L., San Rafael, Marion County, Calif,
BOUGHTON, T. HARRIS, Trenton, N. J.
BOUGHTON, THELMA G., Harrisburg, Pa.
BOUTON, S. MILLES, JR., Lynchburg, Va.
BOYD, EUGENE J., Iowa City, Iowa
BRACKEN, MARK M., Pittsburgh, Pa.
BRADEN, ALBERT H., Houston, Tex.
BRAGDON, JOSEPH, Bethesda, Md.
BRANCH, ARNOLD G., New Brunswick, Canada
BRANCH, CHARLES F., Chicago, Ill.
BRANDES, WALTER W., New York, N. Y.
BRATLEY, FORREST GROVES, Jackson, Miss.
BRAUNSTEIN, WILLIAM P., Weehawken, N. J.
BRAY, WILLIAM E., Charlottesville, Va.
BRILL, ROBERT, Cincinnati, Ohio
BRILMYER, GEORGE J., Aspinwall, Pa.
BRINDLEY, PAUL, Galveston, Tex.
BRINES, OSBORNE A., Detroit, Mich.
BRINKHOUS, KENNETH M., Chapel Hill, N. C.
BRODERS, ALBERT C., Rochester, Minn.
BRODY, HENRY, New York, N. Y.
BROOKS, SAMUEL M., Manchester, N. H.
BRCSIUS, WILLIAM L., Detroit, Mich.
BROWN, ALBERT F., Glendale, Calif.
BROWN, CHESTER RICHARD, New York, N. Y.
BROWN, CLARK E., Philadelphia, Pa.
BROWN, CLAUDE, Philadelphia, Pa.
BROWN, DAVID VAN, Vancouver, Wash.
BROWN, GREGORY N., New York, N. Y.
BROWN, HERBERT R., Rochester, N. Y.
BROWN, H. IVAN, Wilmette. Ill.
BROWN, OMAR JESSE, Washington, D. C.
BROWN, WILBERT O., Pueblo, Colo.
BROWN, WILSON G., St. Louis County, Mo.
BRUECKEN, ALBERT J., Pittsburgh, Pa.
BUCHANAN, EDWIN G., Missoula, Mont.
BUCHER, CARL J., Philadelphia, Pa.
BUCK, LEONARD W., Berkeley, Calif.
BUCKLIN, ROBERT, Saginaw, Mich.
BUDD, JOHN W., Los Angeles, Calif.
BUHLER, VICTOR B., Kansas City, Mo.
BULLARD, H. HAYS, Rochester, N. Y.
BUNTING, HENRY, New Haven, Conn.
BURN, CASPAR G., Brooklyn, N. Y.
BURNS, EDWARD L., Toledo, Ohio
BURT, ROBERT C., Meadville, Pa.
BUSH, J. D., JR., Gadsden, Ala.
BUTCHER, JAMES, Philadelphia, Pa.
BUTLER, WILLIS P., Shreveport, La.
BUTT, EDWARD MERCHANT, Los Angeles, Calif.
BUTTERFIELD, PAUL W., Hagerstown, Md.
BUTTLES, ERNEST H., Burlington, Vt.
BYRNES, ELIZABETH W., Newark, N. J.
BYRNES, THOMAS H., Charlotte, N. C.

CAES, HENRY J., New York, N. Y.
CAIRNS, A. B., Dallas, Tex.
CAJIGAS, THOMAS, Washington, D. C.
CALDWELL, JANET A., Dallas, Tex.
CALLAHAN, WILLIAM P., JR., Wichita, Kan.
CALLENDER, GEORGE R., Washington, D. C.
CAMERO, ANTHONY R., Los Angeles, Calif.
CANAVAN, MYRTELLE M., Boston, Mass.
CANNON, PAUL R., Chicago, Ill.
CARDWELL, EDWARD S., JR., Alexandria, La.
CARES, REUBEN, Wingdale, N. Y.
CARLQUIST, J. H., Salt Lake City, Utah
CARNES, WILLIAM H., Baltimore, Md.
CARPENTER, COY C., Winston-Salem, N. C.
CARR, JESSE L., San Francisco, Calif.
CARRERA, GUILLERMO M., New Orleans, La.
CARSON, PAUL CLARK, Aurora, Colo.
CARTER, CHARLES F., Dallas, Tex.
CARTER, JOHN ROBERT, Iowa City, Iowa
CASALE, WALTER, Worcester, Mass.
CASE, EUGENE A., Philadelphia, Pa.
CASE, LUCIUS W., Pomona, Calif.
CASEY, ALBERT E., Birmingham, Ala.
CASSILLI, ARTURO R., Mount Pleasant, N. J.
CASTLEMAN, BENJAMIN, Boston, Mass.
CATRON, LLOYD, Akron, Ohio
CEFALU, VICTOR, Seattle, Wash.
CHAMBERLIN, ELIZABETH, Bartlesville, Okla.
CHAMBERLIN, WILLIAM B., JR., Cleveland, Ohio
CHAPMAN, BERNARD M., Chicago, Ill.
CHAPMAN, IRVING, Long Island City, N. Y.
CHAPPELL, R. H., Houston, Tex.
CHESNER, CHARLES, Lorain, Ohio
CHIPPS, HENRY DAVIS, Seattle, Wash.
CHOISSER, ROGER M., Washington, D. C.
CHOMET, BERNHARD, Elyria, Ohio
CHRISTIANSON, OSCAR O., Spokane, Wash.
CHRISTOPHER, JAMES G., Detroit, Mich.
CHURG, JACOB, Paterson, N. J.
CICONNE, EMMET F., Philadelphia, Pa.
CLARK, EUGENE, New York, N. Y.
CLARK, GEORGE A., Scranton, Pa.
CLARK, HARRY L., Detroit, Mich.
CLARK, HERBERT CHARLES, Panama, R. de P.
CLARK, JEFFERSON H., Philadelphia, Pa.
CLARKE, B. EARL, New York, N. Y.
CLARKE, JAMES Y., Sioux Falls, S. D.
CLAWSON, BENJAMIN J., Minneapolis, Minn.
CLEMMER, JOHN J., Albany, N. Y.
COBLENZ, AARON, New York, N. Y.
COCHEU, LINDSLEY F., New York, N. Y.
COHEN, FRANK, Quincy, Ill.
COHEN, HILLIARD, New York, N. Y.
COHEN, MORTIMER, Pittsburgh, Pa.
COHEN, SAMUEL E., Elmira, N. Y.
COLE, RUSSEL E., Muncie, Ind.
COLEMAN, FRANCIS C., Des Moines, Iowa
COLLIER, WILLIAM D., Youngstown, Ohio
COLLINS, JOSEPH O., Waterbury, Conn.
COLVIN, MERLE G., Williamsport, Pa.
COLVIN, SAMUEL H., JR., Baton Rouge, La.
COMFORTH, ELIZABETH L., Louisville, Ky.
CONNELL, JOHN H., New Orleans, La.
CONNERTY, HAROLD V., Fall River, Mass.
CONNERY, JOSEPH E., New York, N. Y.

CONTI, HORACE, Chicago, Ill.
COOK, HUNTER S., Philadelphia, Pa.
COOK, WARD H., Brooklyn, N. Y.
COPE, HENRY E., Lansing, Mich.
COPE, THOMAS A., JR , Philadelphia, Pa.
COPELAND, GLENN J., Clifton Springs, N. Y.
CORPER, HARRY J., Denver, Colo.
CORRIGAN, MARION C., Chicago, Ill.
COSTA-MANDRY, OSCAR, Ponce, Puerto Rico
COSTICH, CHARLES W., Hines, Ill.
COULTER, W. W., SR., Houston, Tex.
COX, ALVIN J., San Francisco, Calif.
COX, MARCUS E., Waterbury, Conn.
CRAHAN, THOMAS FRANCIS, San Francisco, Calif.
CRAIG, HELEN F., Boise, Idaho
CRANE, A. R., Philadelphia, Pa.
CRAWFORD, BAXTER, Philadelphia, Pa.
CREED, J. WOODSON, Twin Falls, Idaho
CROLL, DIANE, Chicago, Ill.
CROMARTIE, WILLIAM J., McKinney, Tex.
CROSS, K. R., Des Moines, Iowa
CRYNES, SYLVESTER, Portland, Ore.
CUFF, J. R., Nashville, Tenn.
CULBERTSON, CARL S., South Bend, Ind.
CULBERTSON, CLYDE G , Indianapolis, Ind.
CULL, VIRGINIA L., San Francisco, Calif.
CULLISON, CHARLES W., Chicago, Ill.
CUMMINS, WILLIAM T., San Francisco, Calif.
CUNNINGHAM, JOSEPH A., Birmingham, Ala.
CURPHEY, THEODORE J., Hempstead, N. Y.
CURTIS, GILBERT DEWITT, Glendale, Calif.
CURTIS, STEPHEN H., Troy, N. Y.
CUSTER, R. PHILIP, Philadelphia, Pa.
CUTTINO, JOHN T., Durham, N. C.

DALE, CHARLES L., Los Angeles, Calif.
DALLDORF, GILBERT, Albany, N. Y.
DALRYMPLE, SIDNEY C., Boston, Mass.
DARDINSKI, VINCENT J., Washington, D. C.
DARLINGTON, CHARLES G., New York, N. Y.
DART, RAYMOND O., Washington, D. C.
D'AUNOY, JOSEPH R., New Orleans, La.
DAVENPORT, HERBERT, Houston, Tex.
DAVIDSOHN, ISRAEL, Chicago, Ill.
DAVIDSON, HORACE B., Columbus, Ohio
DAVIS, A. HOBSON, Paterson, N. J.
DAWSON, JAMES R., Nashville, Tenn.
DEADMAN, WILLIAM JAMES, Ontario, Canada
DEANGELIS, CARNELA E., Washington, D. C.
DECOURSEY, ELBERT, Fort Sam Houston, Tex.
DEGROAT, ALBERT, Kalamazoo, Mich.
DEMONBREUN, WILLIAM, Nashville, Tenn.
DENNIS, ROBERT L., San Jose, Calif.
DENTON, JAMES W., New Rochelle, N. Y.
DEPONS, ISABEL S., Asbury Park, N. J.
DE RIVAS, DAMASO, Landsowne, Pa.
DERRICK, W. A., Asheville, N. C.
DESANTO, DOMINIC A., San Diego, Calif.
DESSAU, FREDERICK I., Nyack, N. Y.
DEVEER, J. ARNOLD, East Williston, N. Y.
DEWAN, CHARLES H., Sayre, Pa.
DIAMOND, ISRAEL, Boston, Mass.
DICK, FRED, JR., Augusta, Ga.
DICK, HERMAN J., Syracuse, N. Y.
DICKSON, JAMES GILLESPIE, Durham, N. C.
DIGGS, L. W., Memphis, Tenn.
DIXON, HAROLD M., Washington, D. C.
DOBOS, EMERIC I., Denver, Colo.

DOCHAT, GEORGE R., Akron, Ohio
DOLGOPOL, VERA B., New York, N. Y.
DOMINGUEZ, RAFAEL, Cleveland, Ohio
DONNELLY, ANDREW J., Philadelphia, Pa.
DORGELOH, JUSTIN R., San Francisco, Calif.
DOWLING, GEORGE, Alexandria, Va.
DRAKE, CHARLES R., Minneapolis, Minn.
DRAPIEWSKI, JOHN F., Wilkes-Barre, Pa.
DREYFUSS, MARTIN, Clifton Forge, Va.
DUBIN, ISADORE N., Memphis, Tenn.
DUBLIN, WILLIAM B., Indianapolis, Ind.
DUCEY, E. F., Grand Rapids, Mich.
DUNBAR, FRANK H., Boston, Mass.
DUNLAP, CHARLES E., New Orleans, La.
DUNLOP, JOSEPHINE N., Austin, Tex.
DUNN, ROBERT C., Baltimore, Md.
DUNN, THELMA B., Bethesda, Md.
DURLACHER, STANLEY H., Edgewood, Md.
DUTRA, FRANK R., Cincinnati, Ohio
DUVAL, CHARLES W., San Jose, Calif.
DWYER, JOHN E., Springfield, Mass.
DYRENFORTH, LUCIEN Y., Jacksonville, Fla.

EATON, ARTHUR WALLACE, San Diego, Calif.
EBERSON, FREDERICK, Memphis, Tenn.
EDMEADES, DONALD T., Los Angeles, Calif.
EDMONDS, HENRY W., Seattle, Wash.
EDMONDSON, HUGH A., Los Angeles, Calif.
EDWARDS, JESSE E., Rochester, Minn.
EGGERS, HAROLD E., Omaha, Neb.
EGGSTON, ANDREW A., New York, N. Y.
EHRENREICH, THEODORE, New York, N. Y.
EHRICH, WILLIAM E., Philadelphia, Pa.
EHRLICH, JOSEPH C., Wellfleet, Mass.
EICHORN, KARL B., San Francisco, Calif.
EICHWALD, ERNST J., Boston, Mass.
EIMAN, JOHN, Abington, Pa.
EISEN, J. MILTON, New York, N. Y.
EISENSTEADT, WERNER F., Chicago, Ill.
EITZEN, OLIVER, Cleveland, Ohio
ELLIS, RALPH C., Ogden, Utah
ELTON, NORMAN W , Newton Highlands, Mass
ENDICOT, K. M., Bethesda, Md.
ENZER, NORBERT, Milwaukee, Wis.
ERICKSON, CYRUS, Durham, N. C.
ERNST, K. F., Washington, D. C.
EROS, GEDEON, New York, N. Y.
EVANS, IRA C., St. Petersburg, Fla.

FALISI, J. VINCENT, Washington, D. C.
FALKENSTEIN, A. P., Salem, Ohio
FALKINBURG, L. W., Collingswood, N. J.
FALLAS-DIAZ, MARCIAL, San Jose, Costa Rica
FANGER, HERBERT, Boston, Mass.
FARBER, SIDNEY, Boston, Mass.
FARINACCI, CHARLES J., New York, N. Y.
FEIGIN, IRWIN, Forest Hills, N. Y.
FEIN, MAXWELL J., New York, N. Y.
FELSEN, JOSEPH, New York, N. Y.
FENDRICK, EDWARD, East Orange, N. J.
FENNEL, ERIC A., Honolulu, Hawaii
FENSTERMACHER, RICHARD H., Vicksburg, Miss.
FENTON, CLEMENT C., Morgantown, W. Va.
FERGUSON, FRANKLIN F., Portland, Me.
FERGUSON, J. HOWARD, Syracuse, N. Y.
FERGUSON, JOHN A., Burlington, Vt.
FERGUSON, RALPH L., Vermillion, S. D.
FERMAN-NUNEZ, MARCOS, Milledgeville, Ga.

FERRARO, LOUIS R., Poughkeepsie, N. Y.
FERRIS, HENRY W., Ithaca, N. Y.
FETTERMAN, GEORGE H., Pittsburgh, Pa.
FIDLER, ROSWELL S., Columbus, Ohio
FIENBERG, ROBERT, Framingham, Mass.
FINK, A. A., Topeka, Kan.
FINK, HAROLD, Brooklyn, N. Y.
FISHBACK, HAMILTON, Berkley, Calif.
FISHER, H. RUSSEL, Los Angeles, Calif.
FISHER, JESSIE WESTON, Middletown, Conn.
FISHKEN, B. G., Los Angeles, Calif.
FITE, FRANKLIN, Philadelphia, Pa.
FITZGERALD, PATRICK J., New York, N. Y.
FLASHMAN, DAVID H., Foxboro, Mass.
FLEISHER, MOYER, St. Louis, Mo.
FLORY, CURTIS M., Bennington, Vt.
FLUCK, DAVID A., Trenton, N. J.
FLYNN, JOSEPH E., New York, N. Y.
FOLDES, JULIUS, Hazleton, Pa.
FOLLIS, R. H., Baltimore, Md.
FOORD, ALVIN G., Pasadena, Calif.
FOOT, N. CHANDLER, New York, N. Y.
FOOTE, EDWARD L., Walla Walla, Wash.
FOOTE, FRANK W., New York, N. Y.
FORBUS, WILEY D., Durham, N. C.
FORD, JOHN L., Green Bay, Wis.
FORRESTER, JAMES S., Harrisburg, Pa.
FORRY, FRANK, Indianapolis, Ind.
FOSKETT, HERBERT H., Portland, Ore.
FOSTER, LEE N., Chamblee, Ga.
FOSTER, MILES, Omaha, Nebr.
FOUCAR, FREDERICK H., New York, N. Y.
FOWLER, KENNETH, Houston, Tex.
FOX, HERBERT, Philadelphia, Pa.
FOX, LESTER M., Brooklyn, N. Y.
FOX, ROBERT A., Ridgefield, Conn.
FRANTZ, VIRGINIA K., New York, N. Y.
FREEMAN, WILLIAM, Fall River, Mass.
FREIMAN, DAVID G., Boston, Mass.
FRENCH, A. JAMES, Ann Arbor, Mich.
FRERICHS, JOHN B., St. Louis, Mo.
FRESHMAN, ALEXANDER, Denver, Colo.
FRUEND-KLEMPERER, MARGIT, New Rochelle, N. Y.
FRIEDLAND, LESTER M., Brooklyn, N. Y.
FRIEDMAN, MELVIN, San Francisco, Calif.
FRIEDMAN, NATHAN B., Los Angeles, Calif.
FRIEDRICHS, ANDREW V., New Orleans, La.
FRISCH, ARTHUR W., Portland, Ore.
FROIO, GREGORY F., Philadelphia, Pa.
FROST, THOMAS T., Tucson, Ariz.
FULLER, RALPH H., Phoenix, Ariz.
FULSTOW, MARJORIE, Fairfield, Conn.
FUNK, ERWIN D., Reading, Pa.
FUNKE, JOHN, Atlanta, Ga.
FUREY, ELLEN D., Beaumont, Tex.
FURRER, EMIL D., Eugene, Ore.
FURTH, JACOB, Dallas, Tex.

GALL, EDWARD A., Cincinnati, Ohio
GALLAVAN, ELLA MAE, Pueblo, Colo.
GANNON, JOHN R., Passaic, M. J.
GARBER, C. ZENT, New York, N. Y.
GARDNER, LAWRENCE W., Detroit, Mich.
GARDNER, LOUISE TRENT, Troy, N. Y.
GARROW, IRENE, Astoria, N. Y.
GASPER, ISTVAN A., Bethlehem, Pa.
GATES, OLIVE, Brookline, Mass.
GAULT, EDWIN S., Philadelphia, Pa.
GAY, DOUGLAS M., Wilmington, Del.
GEEVER, ERVING F., Colorado Springs, Colo.
GEIB, WAYNE A., Rapid City, S. D.
GEIGER, JACOB, New York, N. Y.
GEISS, MILLIE A., Philadelphia, Pa.
GENTZKOW, GLEON J., Phoenixville, Pa.
GERBER, ISADORE E., New York, N. Y.
GERMAIN, WILLIAM McK., Cincinnati, Ohio
GERZTL, BRUNO, Oakland, Calif.
GESCHICKTER, CHAS. F., Baltimore, Md.
GETTINGER, RAYMOND, Flushing, N. Y.
GEWANTER, AARON, Somerville, N. J.
GHARPURE, VITHAL VISHVANATH, Bombay, India
GICHNER, MANUEL G., Topeka, Kan.
GIFFEN, HORACE K., Youngstown, Ohio
GILL, ATTICUS J., Dallas, Tex.
GILLETTE, P. C., Sharon, Pa.
GILMORE, HUGH R., Fort George G. Meade, Md.
GINSBURG, GERSHON, Philadelphia, Pa.
GINZLER, ARTHUR M., New York, N. Y.
GIORDANO, A. S., South Bend, Ind.
GITLITZ, ABRAHAM J., Jersey City, N. J.
GLADSTONE, SIDNEY A., New York, N. Y.
GLENN, ROBERT A., Oakland, Calif.
GLIDDEN, H. SPENCER, Andover, Mass.
GOFORTH, JOHN LAWRENCE, Dallas, Tex.
GOLDBERG, SAMUEL, Newark, N. J.
GOLDBLATT, HARRY, Los Angeles, Calif.
GOLDEN, ALFRED, Memphis, Tenn.
GOLDENBURG, MORRIS, Chicago, Ill.
GOMORI, GEORGE, Chicago, Ill.
GONZALES, THOMAS A., New York, N. Y.
GOODALE, RAYMOND H., Worcester, Mass.
GOODMAN, LOUIS, Howard, R. I.
GOODOF, IRVING I., Auburn, Me.
GOODPASTOR, WILLIAM E., Newcastle, Pa.
GOODSITT, EDWARD, Cleveland, Ohio
GORDON, BENJAMIN S., Brooklyn, N. Y.
GORDON, HAROLD, Louisville, Ky.
GORDON, LOUIS Z., Rochester, N. Y.
GORE, IRA, Washington, D. C.
GOSE, A. C., Memphis, Tenn.
GOTTLIEB, JULIUS, Lewiston, Me.
GOTWALD, DAVID K., Nashville, Tenn.
GOULD, SYLVESTER E., Eloise, Mich.
GRADY, HUGH G., Washington, D. C.
GRAEF, IRVING, New York, N. Y.
GRAHAM, ALLEN, Pittsburgh, Pa.
GRAMS, LESLIE R., Chicago, Ill.
GRAUER, ROBERT C., Pittsburgh, Pa.
GRAY, SAMUEL H., St. Louis, Mo.
GRAYZEL, DAVID M., Brooklyn, N. Y.
GREEN, PHILIP P., Washington, D. C.
GREENE, RALPH C., Martinsburg, W. Va.
GREENWOOD, EUNICE S., Louisville, Ky.
GREGORY, JOHN E., Philadelphia, Pa.
GREINER, DANIEL J., Memphis, Tenn.
GRETHMANN, WOLFGANG, Brooklyn, N. Y.
GRILL, JOHN C., Milwaukee, Wis.
GRIMM, HAROLD A., Chicago, Ill.
GRISHMAN, EDITH, New York, N. Y.
GROSS, PAUL, Pittsburgh, Pa.
GROSS, STANLEY, Mount Vernon, N. Y.
GRUENWALD, LOTTE, New York, N. Y.
GRUENWALD, PETER, Brooklyn, N. Y.
GUNN, FRANCIS D., Salt Lake City, Utah
GUNTER, JUNE U., Durham, N. C.
GUTTMAN, PAUL HENRY, Sacramento, Calif.

HAENTZE, FREDERICK E., Cynwyd, Pa.
HAGEBUSCH, OMER E., St. Louis, Mo.
HAGERTY, C. S., Chicago, Ill.
HALA, WILLIAM W., Monroe, N. Y.
HALL, ERNEST M., Los Angeles, Calif.
HALL, W. E. B., St. Louis, Mo.
HALL, WM. M., Bremerton, Wash.
HALLEY, HENRY H., Alton, Ill.
HALPERT, BELA, Oklahoma City, Okla.
HAMILTON, ROBERT C., Pittsburgh, Pa.
HAMILTON, TOM R., Kansas City, Kan.
HAMMACK, ROY W., Los Angeles, Calif.
HANAN, ERNEST B., Springfield, Mo.
HANSEN, HARRY, Plainfield, N. J.
HANSMANN, GEORGE H., Milwaukee, Wis.
HANSON, SAMUEL, Alberta, Canada
HARMOS, OSCAR, Cleveland, Ohio
HARRIS, ARTHUR E., Auburn, N. Y.
HARRIS, PAUL N., Indianapolis, Ind.
HARRIS, WILLIAM H., New Orleans, La.
HARSH, ROBERT C., Pontiac, Mich.
HART, MAYNARD S., El Paso, Tex.
HARTLEY, GEORGE, JR., San Diego, Calif.
HARTMAN, FRANK W., Detroit, Mich.
HARTWELL, R. M.,New Orleans, La.
HASLEY, DANIEL E., Detroit, Mich.
HASTINGS, LOUIS P., Hartford, Conn.
HASTINGS, WILLARD S., Philadelphia, Pa.
HATHAWAY, BURR M., *Mansfield, Ohio*
HAUKOHL, ROBERT S., Milwaukee, Wis.
HAUSE, WELLAND A., El Paso, Tex.
HAUSER, GEORGE H., New Orleans, La.
HAWKINS, WILLIAM BRUCE, Rochester, N. Y.
HAWN, CLINTON VAN ZANDT, Cooperstown, N. Y.
HAWTHORNE, JOHN J., San Francisco, Calif.
HAYES, JOHN J., Albany, N. Y.
HAYMAKER, WEBB, Washington, D.C.
HAYMOND, JOSEPH L.,Indianapolis, Ind.
HAYTHORN, SAMUEL R., Pittsburgh, Pa.
HAZARD, JOHN B., Cleveland Heights, Ohio
HEBBEL, ROBERT, Minneapolis, Minn.
HEBERT, LOUIS A., Lake Charles, La.
HEIBNER, WINSTON C., Baton Rouge, La.
HEID, GEORGE J., Lancaster, Pa.
HEITZMAN, HOLLY H., Green Bay, Wis.
HELLER, E. L., Pittsburgh, Pa.
HELLWIG, CHRISTIAN A., Wichita, Kan.
HELMBOLD, THEODORE R , Pittsburgh, Pa.
HELPERN, MILTON, New York, N. Y.
HELWIG, ELSON B., Washington, D. C.
HELWIG, FERDINAND C., Kansas City, Mo.
HEMSATH, F. A., Lima, Ohio
HENDERSON, DONALD G., Canton, Ohio
HENRY, JAMES W., Evanston, Ill.
HENTEL, WILLIAM, Legion, Tex.
HENTHORNE, J. C., New Orleans, La.
HEPLER, OPAL, Chicago, Ill.
HERBUT, PETER A., Philadelphia, Pa.
HERR, BAYARD S., JR., Amsterdam,N. Y.
HERRMANN, WALTER W., Oakland, Calif.
HERSHBERGER, LLOYD, San Angelo, Tex.
HERSOHN, WILLIAM W., Fort Sam Houston, Tex.
HERTZOG, AMBROSE J., New Orleans, La.
HERZBERG, MORTIMER, Wilmington, N. C.
HICKS, SAMUEL P., Boston, Mass.
HIESCH, EDWIN F., Chicago, Ill.
HIGGINS, GEORGE K., New York, N. Y.
HIGHMAN, BENJAMIN, Silver Springs, Md.
HILDEBRAND, EUGENE, Great Falls, Mont.
HILL, JOSEPH M., Dallas, Tex.
HILL, LEWIS R., Oak Park, Ill.
HILLMAN, OLIVER S., New York, N. Y.
HINERMAN, DORIN L., Ann Arbor, Mich.
HIRSCH, EDWIN F., Chicago, Ill.
HIRSCH, LOUIS, Tucson, Ariz.
HIRST, ALBERT E., JR., Los Angeles, Calif.
HOBBS, MILFORD L., Morgantown, W. Va.
HOBBS, ROBERT E., Shenandoah, Pa.
HOCHMAN, CHARLES H., New York, N. Y.
HODGES, FRANK C., Huntington, W. Va.
HOGE, SOLOMON F., Fayetteville, Ark
HOLLANDER, DAVID H., Baltimore, Md.
HOLLER, HERBERT B., Huntington, L. I., N. Y.
HOLLINGSWORTH, I.PEMBERTON P., Chester, Pa.
HOLMAN, RUSSELL L., New Orleans, La.
HOLT, RUFUS L., Washington, D. C.
HOLYOKE, JOHN B., Hanover, N. H.
HOOKER, JOHN W., Wilmington, Del.
HOPPS, HOWARD C., Oklahoma City, Okla.
HORN, ROBERT C., JR., Philadelphia, Pa.
HORNEFF, JOSEPH A., Philadelphia, Pa.
HOSPERS, CORNELIUS A., Chicago, Ill.
HOUGH, PERRY T., Hartford, Conn.
HOUGHTON, JOHN D., Newton Center, Mass.
HOUSE, REGINALD K., Hanover, N. H.
HOWARD, JOHN W., Wilmington, Del.
HOWE, JOHN S., Brooklyn, N. Y.
HOWELL, KATHERINE M., Chicago, Ill.
HOXIE, EDWIN G., Loma Linda, Calif.
HOYT, LESTER H., Indianapolis, Ind.
HUEPER, WILHELM C., Bethesda, Md.
HULETT, ALBERT G., East Orange, N. J.
HUMMER, GEORGE J., Santa Monica, Calif.
HUMPHREY, ARTHUR A., Battle Creek, Mich.
HUMPHREYS, ELEANOR M., Chicago, Ill.
HUNT, HENRY F., Danville, Pa.
HUNT, HOMER H., Palo Alto, Calif.
HUNTER, FRANK P., Lafayette, Ind.
HUNTER, MARY P., Springfield, Ohio
HUNTER, OSCAR B., Washington, D. C.
HUNTER, OSCAR B., JR., Washington, D. C.
HUNTER, WARREN C., Portland, Ore.
HUNTINGTON, ROBERT W., JR., Los Angeles, Calif.
HYLAND, C. M., Los Angeles, Calif.
HYMAN, MALCOLM A., Brooklyn, N. Y.

IKEDA, KANO, St. Paul, Minn.
INDA, FRANK A., Mineola, N. Y.
INGLEBY, HELEN, Philadelphia, Pa.
IVERSON, LALLA, Chengtu Szechuan, China

JACOBS, WILLIAM F., Buffalo, N. Y.
JACOBSON, MOSES A., Downey, Ill.
JACOBSON, SHELDON ALBERT, Brooklyn, N. Y.
JAESCHKE, W. H., Madison, Wis.
JAFFE, HENRY L., New York, N. Y.
JAMES, ELLERY M., Minneapolis, Minn.
JAMES, WENDELL E., Laconia, N. H.
JENNEY, FLORENCE S., Philadelphia, Pa.
JENNINGS, WILLIAM P., Reading, Pa.
JENSEN, CLYDE R., Seattle, Wash.
JERMSTAD, ROBERT J., Flint, Mich.
JESSUP, DAVID S. D., New York, N. Y.
JETTER, WALTER W., Boston, Mass.
JOHNSON, FRANCIS B., Charleston, S. C.
JOHNSON, LENT CLIFTON, JR., Washington, D. C.

JOHNSON, RICHARD, Columbia, Mo.
JOHNSON, V. MARKLIN, West Palm Beach, Fla.
JOHNSTON, ROBERT D., Portland, Ore.
JOLLIFFE, LESLIE S., Lawrence, Mass.
JONES, FRED D., Springfield, Mass.
JONES, HUGH W., Washington, D. C.
JONES, MAURICE L., Kansas City, Mo.
JONES, RUSSELL S., Memphis, Tenn.
JOSEPHY, HERMAN, Chicago, Ill.
JOYCE, FRANCIS W., Pittsburgh, Pa.

KAHLER, JAMES E., Los Angeles, Calif.
KAHN, JOSEPH R., Cleveland, Ohio
KALASHNIKOFF, PAULINE HANDELMAN, Far Rockaway, N. Y.
KANNER, OSCAR, Parkersburg, W. Va.
KANNERSTEIN, MILTON, St. Louis, Mo.
KANTROWITZ, ABRAHAM R., Brooklyn, N. Y.
KAPLAN, LEO, Los Angeles, Calif.
KARSNER, HOWARD T., Cleveland, Ohio
KASPER, JOSEPH A., Detroit, Mich.
KATZ, SAMUEL D., St. Louis, Mo.
KATZENSTEIN, ROLF, Middletown, Conn.
KAUFMANN, WILLIAM, Holyoke, Mass.
KAUMP, DONALD H., Detroit, Mich.
KEAN, BENJAMIN H., New York, N. Y.
KEANE, GEOFFREY P., Salem, Mass.
KEARNS, JERRY JOSEPH, Chicago, Ill.
KEILLER, VIOLET H., Houston, Tex.
KELLER, W. FLOYD, Oklahoma City, Okla.
KELLERT, ELLIS, Schenectady, N. Y.
KELLEY, WILLIAM, Middletown, N. Y.
KELLNER, AARON, New York, N. Y.
KELSO, RICHARD E., Washington, D. C.
KENDALL, RALPH E., Hartford, Conn.
KENNEDY, PATRICK J., Upper Derby, Pa.
KENYON, THOMAS J., St. Paul, Minn.
KERNODLE, J. D., Alexandria, Va.
KERNOHAN, JAMES W., Rochester, Minn.
KERR, GEORGE R., Orlando, Fla.
KERR, RUSSELL W., Kansas City, Mo.
KESCHNER, HAROLD W., College Park, Md.
KESTON, HOMER D., White Plains, N. Y.
KETCHUM, C. W., Valdosta, Ga.
KIARSIS, VICTOR, New Bedford, Mass.
KILBURY, MERLIN J., Little Rock, Ark.
KIM, GAY BONG, Paterson, N. J.
KIMBALL, THEODORE S., Glendale, Calif.
KIMMELSTEIL, PAUL, Charlotte, N. C.
KING, L. S., Chicago, Ill.
KINGRY, CHARLES B., Denver, Colo.
KINNEY, THOMAS D., Cleveland, Ohio
KIRSHBAUM, J. D., Bakersfield, Calif.
KISELIS, JOHN P., Oak Park, Ill.
KLAM, NAJEEB, Monroe, La.
KLEMPERER, PAUL, New York, N. Y.
KLENK, CHARLES L., St. Louis, Mo.
KLINCK, GUSTAVUS H., JR., Washington, D. C.
KLINE, BENJAMIN S., Cleveland, Ohio
KLUGH, GEORGE F., Atlanta, Ga.
KNOLL, WILLIAM V., Duluth, Minn.
KNOX, LEILA C., New York, N. Y.
KNUTTI, RALPH E., Los Angeles, Calif.
KOGAN, VITALI, New York, N. Y.
KOLETSKY, SIMON, Cleveland, Ohio
KONWALDER, B. E., Van Nuys, Calif.
KONZELMANN, FRANK W., Atlantic City, N. J.
KOPEL, MOSES, New York, N. Y.
KOPPISCH, ENRIQUE, San Juan, Puerto Rico
KOSINSKI, ALEXANDER A., Troy, N. Y.
KOSKY, ALFRED A., Santa Monica, Calif.
KOSTER, E. FREDERICK, Clevaland, Ohio
KOUCKY, RUDOLPH W., Minneapolis, Minn.
KRACKE, ROY R., Birmingham, Ala.
KRAKOWER, C. A., Chicago, Ill.
KRAMER, GABRIEL B., Rockford, Ill.
KRAUS, JOHN E., Peoria, Ill.
KRITZLER, ROBERT A., New York, N. Y.
KRIZ, JOSEPH, Richmond, Va.
KRUMBHAAR, EDWARD B., Philadelphia, Pa
KUHNS, DWIGHT M., Louisville, Ky.
KURLAND, STANLEY K., Denver, Colo.
KUZMA, JOSEPH F., Wauwatosa, Wis.
KVITRUD, GILBERT, St. Paul, Minn.

LACY, GEORGE R., Pittsburgh, Pa.
LAEMLE, ROBERT M., Chicago, Ill.
LAFFERTY, CHARLES R., Montgomery, Ala.
LAIPPLY, THOMAS C., Chicago, Ill.
LAKE, G. C., San Francisco, Calif.
LAMB, FREDERICK H., Davenport, Iowa
LAMB, HAROLD H., Braddock, Pa.
LANDE, KURT E., Hamilton, Ohio
LANYON, W. L., East Stroudsburg, Pa.
LAPI, ANGELO, Denver, Colo.
LARIMORE, LOUISE, Greenwich, Conn.
LARKIN, JOHN J., West Roxbury, Mass.
LARKUM, NEWTON W., Easton, Pa.
LARSON, CHARLES P., Tacoma, Wash.
LARSON, LEONARD W., Bismarck, N. D.
LATTES, RAFFAELE, New York, N. Y.
LATTIMORE, JOHN L., Topeka, Kan.
LAWSON, EDWIN H., New Orleans, La.
LEAKE, NOLAN E., Memphis, Tenn.
LEARNER, AARON, Chicago, Ill.
LEARY, OLGA C., Boston, Mass.
LEBOWICH, JOSEPH, Saratoga Springs, N. Y.
LECOMPTE, PHILLIP M., Jamaica Plain, Mass.
LEDERER, ARTHUR, Chicago, Ill.
LEDERER, MAX, Brooklyn, N. Y.
LEFFLER, HARRISON H., Washington, D C.
LEFKOWITZ, LOUIS L., Bronx, N. Y.
LEHMAN, WM. L., Portland, Ore.
LEITCH, C. G., Kansas City, Mo.
LENNON, HERSHEL C., Greensboro, N. C
LEROY, ELIE P., Chicago, Ill.
LEUCHTENBERGER, RUDOLF, Cleveland, Ohio
LEV, MAURICE, Urbana, Ill.
LEVINE, JACOB, New York, N. Y.
LEVINE, VICTOR, Chicago, Ill.
LEVINSON, SAMUEL A., Chicago, Ill.
LEVY, FRITZ, Huntington, W. Va.
LEWIS, JULIAN H., Chicago, Ill.
LIBER, A. F., Temple, Tex.
LICHTENSTEIN, LOUIS, New York, N. Y.
LIDBECK, WM., Salem, Ore.
LIEBER, MARSHALL M., Philadelphia, Pa.
LIEBOW, AVERILL, New Haven, Conn.
LIGHT, FREDERICK W., Baltimore, Md.
LILLIE, RALPH D., Bethesda, Md.
LIND, CARL J., Minneapolis, Minn.
LINDSAY, JANVIER, Washington, D. C.
LINDSAY, S. T., Rochester, N. Y.
LINDSAY, STUART, San Francisco, Calif
LINN, HERMAN J., Dearborn, Mich.

LIPPINCOTT, LEON S., Daytona Beach, Fla.
LIPPINCOTT, S. W., Seattle, Wash.
LISA, JAMES R., New York, N. Y.
LITTLE, H. G., Wheeling, W. Va.
LLEWELLYN, M. B., Minneapolis, Minn.
LOCHHEAD, HARRIE BURTON, Coatesville, Pa.
LOCRICCHIO, JOHN L., Sharon, Pa.
LODER, MARGARET, Rye, N. Y.
LOEFFLER, ERNST, Chicago, Ill.
LOEFFLER, LOUIS, Chicago, Ill.
LOEFFLER, MARGARET, Jamestown, N. Y.
LOESCH, JOHN, Oneonta, N. Y.
LOFSNESS, STANLEY V., Minneapolis, Minn.
LOHR, OLIVER W., Saginaw, Mich.
LONG, ESMOND R., Philadelphia, Pa.
LOONEY, JOSEPH M., West Roxbury, Mass.
LOSLI, ERNEST J., Portland, Ore.
LOSSMAN, ROBERT T., Detroit, Mich.
LOTT, FREDERICK H., Minneapolis, Minn.
LOVE, JOHN W. P., Willow Grove, Pa.
LOWENHAUPT, ELIZABETH, San Francisco, Calif.
LOWY, OTTO J., Charleston, W. Va.
LUBCHENCO, ALEXIS E., Denver, Colo.
LUBITZ, JOSEPH M., Wood, Wis.
LUCKÉ, BULDUIN, Philadelphia, Pa.
LUFKIN, NATHANIEL H., Minneapolis, Minn.
LUND, HERBERT, Cleveland, Ohio
LUND, PAUL K., Seattle, Wash.
LYNCH, FRANK B., JR., Philadelphia, Pa.
LYNCH, KENNETH M., Charleston, S. C.

MACKEEN, ROBERT A. H., New Brunswick, Canada
MACKENZIE, MICHAEL V., Medford, Mass.
MACKLES, ABRAHAM, Brooklyn, N. Y.
MACKRELL, JOHN S., JR., Cleveland, Ohio
MACMAHON, HAROLD E., Boston, Mass.
MADDEN, SIDNEY C., Atlanta, Ga.
MADSEN, MARTHA, Detroit, Mich.
MAGATH, T. B., Rochester, Minn.
MAHER, ALDEA, New Orleans, La.
MAHON, GEORGE S., Knoxville, Tenn.
MAHON, HUGH W., Denver, Colo.
MALDEIS, HOWARD J., Baltimore, Md.
MALLORY, G. KENNETH, Boston, Mass.
MALLORY, TRACY B., Boston, Mass.
MANER, GEORGE D., Los Angeles, Calif
MANHEIMS, PERRY J., New York, N. Y.
MANION, WILLIAM C., Bladensburg, Md.
MANLOVE, CHARLES H., Portland, Ore.
MANN, BERNARD, Boston, Mass.
MANN, FRANK D., Rochester, Minn.
MANNING, ERNEST T., Omaha, Neb.
MANSHARDT, DONALD O., Rockford, Ill.
MARCUSE, PETER M., Houston, Tex.
MARGOLIS, GEORGE, Durham, N. C.
MARGULIS, AARON E., Santa Fe, N. M.
MARINO, EDMUND R., Brooklyn, N. Y.
MARKLEY, LUTHER A., Teaneck, N. J.
MARKOWITZ, BENJAMIN, Bloomington, Ill.
MARKS, SIDNEY, Palm Springs, Calif.
MARRA, JOHN J., Albany, N. Y.
MARSHALL, L. E., Great Neck, N. Y.
MARTEN, M. EDWARD, Brooklyn, N. Y.
MARTENS, VERNON, Philadelphia, Pa.
MARTIN, MARY ELIZABETH, Billings, Mont.
MARTLAND, HARRISON S., Newark, N. J.
MASLON, MORRIS, Glens Falls, N. Y.
MASON, COYE C., Chicago, Ill.
MASON, DAVID G., Seattle, Wash.
MATHEWS, W. R., Shreveport, La.
MATHIAS, ERNEST E. M., Mt. Morris, New York
MATTHEWS, ARTHUR R. K., Rockford, Ill.
MATTHEWS, WARREN B., Atlanta, Ga.
MAUN, MARK E., Detroit, Mich.
MAVRELIS, WILLIAM P., Chicago, Ill.
MAXWELL, ELMER S., Lexington, Ky.
MAYNARD, C. W., Pueblo, Colo.
MAYNER, FREDERICK A., Wyandotte, Mich.
MCBRIDE, W. P., Rutland, Vt.
MCCANDLESS, CARL M., Corona, Calif.
MCCARTER, JOHN C., Evanston, Ill.
MCCARTNEY JAMES S., JR., Minneapolis, Minn.
MCCLELLAN, ROBERT H., Irwin, Pa.
MCCLURE, GEORGE Y., Urbana, Ill.
MCCOLL, CHARLES R., Tacoma, Wash.
MCCULLOUGH, KENDRICK, Valhalla, N. Y.
MCCURDY, GORDON A., Victoria, B. C.
MCCURDY, THOMAS, San Jose, Calif.
MCCUTCHEON, MORTON, Philadelphia, Pa.
MCDONALD, JOHN R., Rochester, Minn.
MCFADDEN, JAMES M., Lafayette, Ind.
MCGARY, LESTER, Madison, Wis.
MCGRAW, JOHN J., Bryn Mawr, Pa.
MCGREW, DONALD, Spartanburg, S. C.
MCJUNKIN, FRANK A., Orlando, Fla.
MCKINLEY, HUGH A., Jacksonville, Ill.
MCLATCHIE, LOLA DELL, Alberta, Canada
MCLEOD, CHRISTIE, Middletown, Conn.
MCMANUS, J. F. A., Birmingham, Ala.
MCMEANS, JOSEPH W., Florence, S. C.
MCNAMARA, WM. L., Hines, Ill.
MCNAUGHT, JAMES B., Denver, Colo.
MCNEIL, CRICHTON, Salt Lake City, Utah
MCPEAK, ELSIE W., Boston, Mass.
MCQUEENEY, ANDREW, Bridgeport, Conn.
MEDLAR, EDGAR M., Mt. Kisco, N. Y.
MEEKER, LOUISE, Scarsdale, N. Y.
MEISSNER, WILLIAM A., Boston, Mass.
MELLON, RALPH R., Pittsburgh, Pa.
MELNICK, PERRY J., Beverly Hills, Calif.
MENDELOFF, JOSEPH, Fayetteville, N. C.
MENLOWE, PATTERSON M., McKeesport, Pa.
MENNE, FRANK R., Portland, Ore.
MENTEN, MAUD L., Pittsburgh, Pa.
MERANZE, DAVID R., Philadelphia, Pa.
MERKEL, WALTER, Baltimore, Md.
MERMOD, CAMILLE, Rochester, Minn.
MEYER, ELIZABETH, Forest Hills, N. Y.
MEYER, LEO M., Brooklyn, N. Y.
MIALE, J. B., Marshfield, Wis.
MICHAEL, AMOS C., Indianapolis, Ind.
MICHAEL, PAUL, Oakland, Calif.
MICHELSON, ISRAEL D., Memphis, Tenn.
MIDER, G. BURROUGHS, Rochester, N. Y.
MILLER, AURA J., Louisville, Ky.
MILLER, RALPH E., Hanover, N. H.
MILLES, GEORGE, Chicago, Ill.
MILLISER, RUSSELL V., Columbus, Ohio
MILLMAN, LOUIS J., New York, N. Y.
MILLS, FRANCES MCCONNELL, Denver, Colo.
MILLS, FRED E., Evansville, Ind.
MILLS, HERBERT R., Tampa, Fla.
MINCKLER, JEFF., Portland, Ore.
MITCHELL, NATHAN, Brooklyn, N. Y.
MOITRIER, WILLIAM, Brooklyn, N. Y.
MONACO, A. RALPH, Washington, D. C.

MONTGOMERY, LALL G., Muncie, Ind.
MOODY, ADELBERT M., San Francisco, Calif.
MOOLTEN, SYLVAN E., Highland Pk., N. J.
MOON, HENRY D., San Francisco, Calif.
MOON, VIRGIL H., Philadelphia, Pa.
MOORE, GERTRUDE, Oakland, Calif.
MOORE, J. J., Chicago, Ill.
MOORE, JOHN M., San Antonio, Tex.
MOORE, MAURICE R., Norwich, Conn.
MOORE, ROBERT A., St. Louis, Mo.
MOORE, ROBERT M., Vicksburg, Miss.
MORAGUES, VINCENT, St. Louis, Mo.
MORAN, CLARENCE S., Mitchell, S. D.
MORAN, T. J., Danville, Va.
MOREHEAD, ROBERT P., Winston-Salem, N. C
MORGAN, DAVID R., Philadelphia, Pa.
MORITZ, ALAN R., Boston, Mass.
MORRIONE, THOMAS G., Burlington, Vt.
MORRIS, JOYCE, Greenwich, Conn.
MORRIS, LLOYD E., Bangor, Me.
MORSE, PLINN F., Detroit, Mich.
MOSS, EMMA S., New Orleans, La.
MOSS, LEO D., New York, N. Y.
MOSS, THOMAS C., Memphis, Tenn.
MOSSBERGER, JOSEPH I., Denver, Colo.
MOSTELLER, RALPH, Beckley, W. Va.
MOSTOFI, FATHOLLAH K., Washington, D. C.
MOTYCA, LAWRENCE J., Norfolk, Va.
MUGRAGE, EDWARD R., Denver, Colo.
MUIRHEAD, E. E., Dallas, Tex.
MULLER, GULLI L., Newtonville, Mass.
MULLIGAN, RICHARD, Denver, Colo.
MULLINS, DEWITT F., JR., Athens, Ga.
MULSOW, FREDERICK, Cedar Rapids, Iowa
MURPHY, GEORGE H., Winchester, Va.
MURPHY, LYMAN C., Wichita, Kansas
MURRAY, NELSON A., Jacksonville, Fla.
MYERS, E. E., Philippi, W. Va.

NAUMANN, HANS N., Jackson, Miss.
NEAL, M. PINSON, Columbia, Mo.
NEDZEL, ALEXANDER J., Chicago, Ill.
NEIDHARDT, H. W., Galveston, Tex.
NEIMAN, BENJAMIN, Chicago, Ill.
NELSON, ARTHUR A., Washington, D. C.
NELSON, WALTER C., Binghamton, N. Y.
NESBIT, ROBERT R., New Haven, Conn.
NETTLESHIP, ANDERSON, Little Rock, Ark.
NEUBUERGER, KARL T., Denver, Colo.
NEUMAN, LESTER, Washington, D. C.
NEWMAN, BENJAMIN, Charleston, W. Va.
NICHOLSON, DANIEL, Winnipeg, Canada.
NICKERSON, DONALD A., Salem, Mass.
NICKSON, DELBERT H., Seattle, Wash.
NOBLE, JOHN F., St. Paul, Minn.
NOLAN, LEWIS E., Fairmont, W. Va.
NORRIS, JACK C., Atlanta, Ga.
NORRIS, ROBERT F., Wynnewood, Pa.
NORTON, LOUISA MACDONALD, Rochester, N. H.
NORWOOD, VERNON H., Baltimore, Md.
NOWAKOVSKY, SOPHIE T., Chicago, Ill.

ODESSKY, LOUIS, Augusta, Ga.
OESTERLIN, ERNST J., Mount Pleasant, Iowa
OFFENKRANTZ, FREDERICK M., Paterson, N. J.
OGDEN, MICHAEL A., Passaic, N. J.
OGILVIE, ORIN A., Salt Lake City, Utah
OGURA, GEORGE I., Denver, Colo.
OLCOTT, CHARLES T., New York, N. Y.
OLDT, MARY RUTH, Oakland, Calif.
OLIVETTI, RENZO G., New York, N. Y
OLKEN, HARRY G., Boston Mass.
OLSEN, RICHARD E., Pontiac, Mich.
OLSON, ERNEST S., Elgin, Ill.
OOSTING, MELVIN, Dayton, Ohio
OPPER, LINCOLN, Torrington, Conn.
ORBISON, J. L., Cleveland, Ohio.
OSBORNE, ROLAND H., Los Angeles, Calif.
OSGOOD, RUDOLF, Lexington, Mass.
OWEN, CLARENCE I., Detroit, Mich.
OWEN, MAY, Ft. Worth, Tex.
OWENS, JAMES N., JR., Shawnee, Okla.

PALIK, EMIL E., Tulsa, Okla.
PALMER, H. D., Denver, Colo.
PALMER, JAY J., Battle Creek, Mich.
PALTAUF, RUDOLF M., New York, N. Y.
PARK, JAMES H., Concord, N. H.
PARKER, FRANCIS P., Charlottesville, Va.
PARKER, FREDERIC, JR., Boston, Mass.
PARKHILL, EDITH M., Rochester, Minn.
PARMACEK, LOUIS, Chicago, Ill.
PARSONS, LAWRENCE, Reno, Nev.
PARSONS, ROBERT J., Oakland, Calif.
PASTERNACK, JOSEPH G., Wichita Falls, Tex.
PATTERSON, JAMES N., Tampa, Fla.
PATTON, FRANK R., Spokane, Wash.
PATTON, MATTHEW M., Spokane, Wash.
PAYNE, C. ALLEN, Grand Rapids, Mich.
PAYNE, F. C., Dayton, Ohio
PEALE, AUGUSTIN R., Philadelphia, Pa.
PEARCE, JOHN MUSSER, New York, N. Y.
PEARSON, BJARNE, Burlington, Vt.
PEASE, GERTRUDE L., Rochester, Minn.
PEASLEY, E. D., Asheville, N. C.
PECKHAM, A. L., Poughkeepsie, N. Y.
PEERS, JAMES H., Bethesda, Md.
PEERY, THOMAS M., Washington, D. C.
PEKELIS, EMANUEL, Sioux Falls, S. D.
PEÑAS, MANUEL, St. Louis, Mo.
PENKE, MADELINE, Corona, N. Y.
PENNELL, S., Alberta, Canada
PERMAR, HOWARD H., Pittsburgh, Pa.
PERRIN, THEODORE L., Bethesda, Md.
PERRY, ISABELLA H., Washington, D. C.
PESSIN, SAMUEL B., Milwaukee, Wis.
PETERSON, RAYMOND F., Butte, Mont.
PHETEPLACE, WILLARD S., Davenport, Iowa
PHILLIPS, CHARLES, Temple, Tex.
PICKARD, RAWSON J., San Diego, Calif.
PIERCE, LYDIA B., Westborough, Mass.
PIETROLUONGO, ANTHONY L., Philadelphia, Pa.
PILCHER, JOHN F., Corpus Christi, Tex.
PILOT, ISADORE, Chicago, Ill.
PILOT, JEAN, Chicago, Ill.
PINCUS, JULIUS, New York, N. Y.
PINKERTON, HENRY, St. Louis, Mo.
PIRANI, CONRAD C., Chicago, Ill.
PIZZOLATO, PHILIP, New Orleans, La.
PLATT, WILLIAM R., Camden, N. J.
PLAUT, ALFRED, New York, N. Y.
PLOWDEN, HENRY H., Columbia, S. C.
POLAYES, SILIK, Brooklyn, N. Y.
POLLACK, ABOU D., Frederick, Md.
POLLAK, OTOKAR JAROSLAV, Arden, Del.
PONS, CARLOS A., Asbury Park, N. J.

Popoff, Nicholas W., Hunt, N. Y.
Popper, Hans, Chicago, Ill.
Poppiti, Robert J., Miami Beach, Fla.
Porro, Francis W., Roanoke, Va.
Porter, Joseph E., Portland, Me.
Porter, Mary McKee, Wayne, Pa.
Portuondo, Bonaventure C., St. Louis, Mo.
Posey, L. D., Birmingham, Ala.
Postoloff, Anthony V., Buffalo, N. Y.
Potter, Edith L., Chicago, Ill.
Potter, Frederick C., Cuyahoga Falls, Ohio
Powell, Elizabeth B., Houston, Tex.
Powell, William N., Temple, Tex.
Pratt, Orlyn B., Los Angeles, Calif.
Pratt-Thomas, H. R., Charleston, S. C.
Prentice, Hazel R., Kalmazoo, Mich.
Preuss, Fred Simon, Batavia, N. Y.
Pribram, Ernest A., Chicago, Ill.
Price, Aaron Sumner, Honolulu, T. H.
Price, H. Preston, Jersey City, N. J.
Priestman, Gordon, Kings Park, N. Y.
Proescher, Frederick, San Jose, Calif.
Proskauer, George S., Akron, Ohio
Pund, Edgar R., Augusta, Ga.
Pusch, Lewis C., York, Pa.
Putschar, Walter G. J., Charleston, W. Va.

Queen, Frank B., Portland, Ore.
Quinland, W. S., Tuskogee, Ala.

Rabson, S. M., Ft. Wayne, Ind.
Ragins, Alex B., Chicago, Ill.
Ragle, Harold E., Palo Alto, Calif.
Rakieten, Morris L., Brooklyn, N. Y.
Ramsey, G. W., Washington, Pa.
Ramsey, Thomas L., Toledo, Ohio
Randall, Charles C., Lexington, Ky.
Randall, William Spears, New Orleans, La.
Rappaport, Henry, Washington, D. C.
Rappoport, Arthur E., Youngstown, Ohio
Ratcliffe, Albert W., Evansville, Ind.
Rathmell, Thomas K., Trenton, N. J.
Raven, Clara, Youngstown, Ohio
Ravid, Jacob M., New York, N. Y.
Rea, Stanley L., Reno, Nev.
Read, William T., Jr., Camden, N. J.
Rees, William T., Philadelphia, Pa.
Rehbock, Donald J., Cleveland, Ohio
Reichle, Herbert S., Stuttgart, Germany
Reifenstein, George H., Syracuse, N. Y.
Reimann, D. L., Baltimore, Md.
Reimann, Stanley P., Philadelphia, Pa.
Reingold, Irving M., Van Nuys, Calif.
Reinhart, Harry L., Columbus, Ohio
Reitz, Charles B., Allentown, Pa.
Reyes, Felix M., San Juan, Puerto Rico
Rhamy, Bonnelle W., Ft. Wayne, Ind.
Rhoden, Alfred E., Toledo, Ohio
Rice, E. Clarence, Washington, D. C.
Rice, Maurice M., Santa Ana, Calif.
Rice, Thurman B., Indianapolis, Ind.
Rice, Walter Gowans, Temple, Tex.
Rich, George T., Toledo, Ohio
Richardson, Howard L., Portland, Ore.
Richardson, Russell, Philadelphia, Pa.
Richter, Maurice N., New York, N. Y.
Ricker, Walter A., Jr., Seattle, Wash.
Rigdon, Raymond H., Galveston, Tex.
Riggs, Helena, Philadelphia, Pa.
Rinehard, James F., San Francisco, Calif.
Rini, Joseph, Springfield, Mass.
Ritchie, Gorton, Milwaukee, Wis.
Robbins, Stanley L., Boston, Mass.
Roberts, James Bratton, Albany, N. Y.
Roberts, James R., St. Louis, Mo.
Rockman, Jacob, Miami, Fla.
Rodger, James Y., Lowell, Mass.
Rogers, Raymond J., Oconto, Wis.
Rohdenburg, George L., New York, N. Y.
Rohow, Fred M., Whittier, Calif.
Rooney, J. Stewart, Boston, Mass.
Rosahn, Paul, New Britain, Conn.
Rose, Salvatore J., Orange, N. J.
Rose, S. Brandt, Philadelphia, Pa.
Rosen, Samuel H., Kew Gardens, N. Y.
Rosenbaum, David, Indianapolis, Ind.
Rosenblatt, Philip, Brooklyn, N. Y.
Rosenthal, Julius, Bronx, N. Y.
Rosenthal, Lazar, Brooklyn, N. Y.
Rosenthal, Sol Ray, Chicago, Ill.
Rosenzweig, Maurice, Milwaukee, Wis.
Ross, Elizabeth, Nova Scotia, Canada
Rothrock, Henry A., West Chester, Pa.
Rothstein, Isadore, New York, N. Y.
Rottino, Antonio, New York, N. Y.
Royce, Clayton, Jacksonville, Fla.
Rubenstein, Victor G., Camp Haan, Calif.
Rubnitz, Abraham, Omaha, Neb.
Rudo, Nathan, Baltimore, Md.
Ruediger, Ernest H., Chattahochee, Fla.
Rukstinat, George J., Chicago, Ill.
Russell, Hollis K., Scarsdale, N. Y.
Russell, William O., Houston, Tex.
Russi, Simon, Richmond, Va.
Russum, Benjamin C., Omaha, Neb.
Rutledge, Enid K., Denver, Colo.
Ryder, Charles T., Colorado Springs, Colo.

Saccone, Andrea, New York, N. Y.
Sacks, Milton, Baltimore, Md.
Sahyoun, Philip, Richmond, Va.
St. George, Armin V., New York, N. Y.
Sala, Angelo M., New York, N. Y.
Sanders, Charles B., Houston, Tex.
Sanes, Samuel, Buffalo, N. Y.
Sanford, Arthur H., Rochester, Minn.
Sano, Machteld E., Philadelphia, Pa.
Santora, Edward, Jackson Heights, N. Y.
Saphir, Otto, Chicago, Ill.
Sappington, S. W., Bryn Mawr, Pa.
Satterlee, Richard C., San Francisco, Calif.
Saxton, John A., St. Louis, Mo.
Saye, Ernest B., Thomasville, Ga.
Sayet, Maxwell M., Miami Beach, Fla.
Sayre, George P., Rochester, Minn.
Schaefer, Leroy W., Lewistown, Pa.
Schafer, Etheldred L., Madison, Wis.
Schattenberg, Herbert J., San Antonio, Tex.
Schelm, G. W., Battle Creek, Mich.
Schenken, John R., Omaha, Neb.
Scherer, John H., Richmond, Va.
Schiller, Walter, Evanston, Ill
Schlademan, K. R., Ft. Wayne, Ind.
Schleifstein, Joseph I., Albany, N. Y.
Schlesinger, Monroe J., Boston, Mass.
Schlumberger, Hans G., Columbus, Ohio

SCHMEISSER, HARRY C., Memphis, Tenn.
SCHMIDT, EDWARD C. H., Kansas City, Mo.
SCHMIDT, J. ROSS, Northfield, N. J.
SCHMITT, PAUL G. F., Chicago, Ill.
SCHNEIDER, HARRY J., French Camp, Calif.
SCHNEIDER, LOUIS A., Ft. Wayne, Ind.
SCHOFIELD, NORMAN D., Galveston, Tex.
SCHRADIECK, CONSTANT E., Erdenheim, Pa.
SCHREK, ROBERT, Hines, Ill.
SCHULTZ, E. W., Stanford, Calif.
SCHULZ, REUBEN Z., Cambridge, Mass.
SCHUSTER, MYRON, Milwaukee, Wis.
SCHWANDER, HOWARD, Little Rock, Ark.
SCOTT, JAMES K., Rochester, N. Y.
SCOTTI, THOMAS, Richmond, Va.
SCOVILLE, HELEN W., Pittsfield, Mass.
SCULLARD, GARNER, Warren, Ohio
SEECOF, DAVID P., Bronx, N. Y.
SEEMAN, W. H., New Orleans, La.
SEGAL, ABRAHAM, Cleveland, Ohio
SELSAM, ETTA B., Terre Haute, Ind.
SELZER, ISIDORE, New York, N. Y.
SEVERANCE, ALVIN O., San Antonio, Tex.
SHACKFORD, BARTLETT C., Palos Verdes Estates, Calif.
SHAPIRO, MORRIS W., Brighton, Mass.
SHARNOFF, J. GEORGE, Mount Vernon, N. Y.
SHAVER, JOHN S., Oakland, Calif.
SHAWEKER, MAX, Canton, Ohio
SHEEHAN, JOHN F., Chicago, Ill.
SHEEHY, JOHN J., Salt Lake City, Utah
SHELDON, WALTER H., Atlanta, Ga.
SHEPEARD, WALTER L., Augusta, Ga.
SHERMAN, FRANK E., JR., Pittsburgh, Pa.
SHERMAN, IRVING, Brooklyn, N. Y.
SHERMAN, MAY, Greystone Park, N. J.
SHERWOOD, NOBLE P., Lawrence, Kan.
SHIMER, WILL, Indianapolis, Ind.
SHIREY, RALPH W., Yakima, Wash.
SHOEMAKER, ROSEMARY, Los Angeles, Calif.
SHULMAN, MURRAY W., Newark, N. J.
SIEBERT, FRANK T., Pittsburgh, Pa.
SIEBERT, WALTER, J., St. Louis, Mo.
SIEGEL, HENRY, Long Island City, N. Y.
SILLIPHANT, WILLIAM M., Bethesda, Md.
SILVERMAN, GERTRUDE, New York, N. Y.
SILVERMAN, I. JEROME, Bronx, N. Y.
SIMARD, ERNEST E., Pacific Grove, Calif.
SIMMONS, J. S., Boston, Mass.
SIMON, MORRIS A., Montreal, Canada
SIMON, RICHARD J., Pittsburgh, Pa.
SIMONDS, JAMES P., Chicago, Ill.
SIMPSON, JOHN C., Norristown, Pa.
SIMPSON, WALTER M., Dayton, Ohio
SINGMAN, DAVID, Berkeley, Calif.
SKINNER, DAVID, Newton Lower Falls, Mass.
SKINSNES, OLAF K., Evanston, Ill.
SLOAN, RUELL A., Arlington, Va.
SMALL, CARROL S., Loma Linda, Calif.
SMETANA, HANS F., Washington, D. C.
SMITH, EDWARD B., Narberth, Pa.
SMITH, H. P., New York, N. Y.
SMITH, J. HOWARD, Portville, N. Y.
SMITH, LAWRENCE W., New York, N. Y.
SMITH, PEARL M., San Francisco, Calif.
SMITH, RALPH P., Halifax, Nova Scotia
SNAVELY, JOHN G., Stamford, Conn.
SNEEDEN, VINTON D., Portland, Ore.
SNEIDERMAN, HARRY, Cleveland, Ohio
SNIFFEN, RONALD C., Boston, Mass.
SOLOMON, CYRIL, New York, N. Y.
SOMMER, MELVIN L., Los Angeles, Calif.
SOPHIAN, LAWRENCE, Staten Island, N. Y
SPAETH, WILLIAM L., Philadelphia, Pa.
SPAIN, DAVID M., New York, N. Y.
SPEER, FRANCIS D., New York, N. Y.
SPENCER, HUGH R., Baltimore, Md.
SPITZ, HENRY, New York, N. Y.
SPITZ, HERMAN, Nashville, Tenn.
SPITZ, SOPHIE, New York, N. Y.
SPRINZ, HELMUTH, Boston, Mass.
SPRUNT, DOUGLAS H., Memphis, Tenn.
SQUIRES, GRETCHEN V., Pensacola, Fla.
STA CRUZ, JUAN Z., Manila, P. I.
STADER, DONALD E., Allentown, Pa.
STANFORD, ADDIE D., New York, N. Y.
STANGL, P.E., St. Cloud, Minn.
STARK, ERNEST, Burlington, Vt.
STARRY, ALLEN C., Sioux City, Iowa
STASNEY, JOSEPH, Philadelphia, Pa.
STEELE, ALBERT E., Roslindale, Mass.
STEEN, HARRY M., Springfield, Ill.
STEER, ARTHUR, Denver, Colo.
STEINBERG, BERNHARD, Toledo, Ohio
STEINER, PAUL E., Chicago, Ill.
STERN, KURT, Chicago, Ill.
STERNBERG, WILLIAM H., New Orleans, La.
STEVENS, DOROTHY L., Johnson City, N. Y.
STEWART, CHARLES W., Baltimore, Md.
STEWART, FRED W., New York, N. Y.
STEWART, HAROLD L., Bethesda, Md.
STEWART, HENRY A., Gettysburg, Pa.
STEWART, JOHN K., Clinton, Iowa
STILLMAN, RALPH G., New York, N. Y.
STOCK, AARON H., Pittsburgh, Pa.
STOFER, BERT E., Wichita, Kan.
STOHR, GRETE, New York, N. Y.
STONE, FRED D., Chicago, Ill.
STONE, MURRAY C., Springfield, Mo.
STOUT, ARTHUR P., New York, N. Y.
STOUT, BEECHER F., San Antonio, Tex.
STOVALL, WILLIAM D., Madison, Wis.
STOWE, W. PARKER, Miami Beach, Fla.
STOWELL, ROBERT E., Kansas City, Kan.
STRASSMAN, GEORGE S., Waltham, Mass.
STRAUS, REUBEN, Los Angeles, Calif.
STRAUSER, E. R., Neenah, Wis.
STRAUSS, ARNOLD F., Norfolk, Va.
STRUMIA, MAX M., Bryn Mawr, Pa.
STRYKER, WALTER A., Detroit, Mich.
STUMP, DAVID J., New York, N. Y.
STUURMANN, S. H., Los Angeles, Calif.
SUMERLIN, HAROLD S., San Diego, Calif.
SUMMERILL, FREDERICK, Newburgh, N. Y.
SUMMERVILLE, WARD W., Kansas City, Kan.
SUNDERMAN, F. WILLIAM, Cleveland, Ohio
SWAN, LAWRENCE L., New Orleans, La.
SWEANY, HENRY C., Chicago, Ill.
SWEETS, HENRY H., JR., Lexington, Ky.
SZANTO, PAUL B., Chicago, Ill.

TAFT, EDGAR B., Boston, Mass.
TANNENBERG, JOSEPH, Batavia, N. Y.
TANNER, FRANK H., Lincoln, Neb.
TANNHAUSER, SIEGFRIED, Buffalo, N. Y.
TARLOW, LILLAN, Chicago, Ill.
TAUB, JACOB, Bronx, N. Y.

TAYLOR, FRANCES M., Philipsburg, Pa.
TAYLOR, J. S., Kingston, N. Y.
TAYLOR, RICHARD C., Cleveland, Ohio
TEDESCHI, CESARE, New York, N. Y. (last address)
TENNANT, ROBERT, Hartford, Conn.
TERRELL, TRUMAN C., Ft. Worth, Tex.
TERRY, BENJAMIN T., Tacoma, Wash.
TESSMER, CARL, APO 500, % Postmaster, San Francisco, Calif.
THARINGER, EDWARD L., Milwaukee, Wis.
THOMAS, A. R., Fort Sam Houston, Tex.
THOMAS, WALTER S., Rochester, N. Y.
THOMAS, WILBUR C., Phildelphia, Pa.
THOMPSON, RALPH L., St. Louis, Mo.
THOMPSON, R. M., Washington, D. C.
THORNESS, EDWIN T., Dubuque, Iowa
THORNTON, HAROLD C., Indianapolis, Ind.
THURSTON, ERIC W., Chicago, Ill.
TIGERTT, WILLIAM D., Washington, D. C.
TILDEN, IRVIN L., Honolulu, T. H.
TOCANTINS, LEANDRO M., Philadelphia, Pa.
TODD, LESTER C., Charlotte, N. C.
TOLLMAN, JAMES P., Omaha, Neb.
TOLSTOI, GEORGE, Uniontown, Pa.
TOMLINSON, WRAY J., Ft. Wayne, Ind.
TOMPKINS, VICTOR N., Albany, N. Y.
TOOLEY, GEORGE E., Seattle, Wash.
TOWNSEND, ELEANOR, Louisville, Ky.
TOWNSEND, FRANK M., Harlingen, Tex.
TRAGERMAN, L. J., Los Angeles, Calif.
TRIBBY, WILLIAM W., *Memphis, Tenn.*
TROLL, MARY MAHER, Pittsburgh, Pa.
TRUMBULL, MERLIN L., Memphis, Tenn.
TRUMPER, ABRAHAM, Montgomery, Ala.
TRYGSTAD, REIDAR, Central Islip, N. Y.
TULLIS, JOHN L., Bethesda, Md.
TUTA, JOSEPH A., Long Beach, Calif.
TUTHILL, C. R., Wilkes-Barre, Pa.

UNGAR, JOHN, Sewickley, Pa.
UPSHER, ALBERT EDWARD, Kansas City, Mo.

VALDES-DAPENA, ANTONIO M., Darby, Pa.
VALENTINE, ELEANOR H., Denver, Colo.
VANAUKEN, HOWARD A., Ft. Sam Houston, Tex.
VANCE, BENJAMIN M., New York, N. Y.
VANDEGRIFT, EARL, Wilkinsburg, Pa.
VANDEGRIFT, WILLIAM B., Baltimore, Md.
VAN HECKE, LEANDER J., Milwaukee, Wis.
VAN HORN, HERMAN H., Jordan, N. Y.
VAN PERNIS, PAUL V., Grand Rapids, Mich.
VASS, ALAJOS, Springfield, Ill.
VENABLE, DOUGLAS R., Columbus, Ga.
VIDOLI, MARINO F., Toledo, Ohio
VOGEL, PETER, New York, N. Y.
VOLK, BRUNO W., Chicago, Ill.
VON GLAHN, WILLIAM C., New York, N. Y.
VON HAMM, EMMERICH, Columbus, Ohio
VORDER BRUEGGE, COLIN F., Washington, D. C.
VORWALD, ARTHUR J., Saranac Lake, N. Y.

WACHSTEIN, MAXIMILIAN, Brooklyn, N. Y.
WADSWORTH, RICHARD C., Bangor, Me.
WAGNER, JOHN A., Baltimore, Md.
WAHL, HARRY R., Kansas City, Kan.
WALKER, GEORGE A., Oakland, Calif.
WALKER, GERALD C., Utica, N. Y.
WALKER, JAMES W., Washington, D. C.
WALKER, THOMAS F., Great Falls, Mont.
WALKER, THOMAS T., Watertown, N. Y.
WALL, WILLIAM A., Cortland, N. Y.
WALLACE, STUART A., Houston, Tex.
WALTZ, ARTHUR D., Philadelphia, Pa.
WANSTROM, RUTH C., Ann Arbor, Mich
WARNER, CARROL G , Baltimore, Md.
WARNER, EMORY D., Iowa City, Iowa
WARREN, *SHIELDS, Boston, Mass.*
WARTMAN, WILLIAM B., Chicago, Ill.
WARWICK, MARGARET, Buffalo, N. Y.
WASON, ISABEL M., New Bedford, Mass.
WASSERMAN, PHILIP, Cincinnati, Ohio
WATSON, GEORGE A., San Francisco, Calif.
WAUGH, THEODORE R., Montreal, Canada
WEBER, CLARENCE J., Kansas City, Kan.
WEEDON, FREDERICK R., Jamestown, N. Y.
WEEK, LYLE A., Rochester, Minn.
WEETER, HARRY M., Louisville, Ky.
WEINBERG, TOBIAS, Baltimore. Md.
WEINTRAUB, SOLOMON, New York, N. Y.
WEISKOTTEN, HERMAN G., Syracuse, N. Y.
WEISS, CHARLES, Philadelphia, Pa.
WEISS, LEO, Staten Island, N. Y.
WELAND, R. E., Cedar Rapids, Iowa
WELLBROCK, W. L. A., Portsmouth, Va.
WELLER, CARL V., Ann Arbor, Mich.
WELLS, ARTHUR H., Duluth, Minn.
WELLS, BENJAMIN B., Houston, Tex.
WENNER, JOHN J., Allentown, Pa.
WERNE, JACOB, Astoria, N. Y.
WERT, EARL B., *Mobile, Ala.*
WERTHAMMER, SIEGFRIED, Huntington, W. Va.
WESCOTT, ADELINE, Newburgh, N. Y.
WHEELOCK, MARK C., Chicago, Ill.
WHIPPLE, GEORGE H., Rochester, N. Y.
WHITCHER, B. R., New Brunswick, N. J.
WHITE, EARL T., Greenville, Miss.
WHITMORE, EUGENE R., Washington, D. C.
WHORTON, CARL M., New Orleans, La.
WIENER, ALEXANDER S., Brooklyn, N. Y.
WIENER, MORRIS F., Miami Beach, Fla.
WIGLESWORTH, FREDERICK W., Montreal, Canada
WILBUR, ERNEST L., North Little Rock, Ark.
WILDMAN, OTIS, Washington, D. C.
WILENS, SIGMUND, New York, N. Y.
WILLIAMS, GEORGE Z., Richmond, Va.
WILLIAMS, JOHN W., Louisville, Ky.
WILLIAMS, ONIE O., Phoenix, Ariz.
WILLIAMS, ROBERT J., Providence, R. I.
WILLIAMS, THOMAS H., Manitoba, Canada
WILSON, HUGH, Highland Park, Ill.
WILSON, M. W., Mountain Home, Tenn.
WINSHIP, THEODORE, Washington, D. C.
WISE, I. *MILTON, Mobile, Ala.*
WOLBACH, S. B., Boston, Mass.
WOLF, ABNER, New York, N. Y.
WOLL, EPHRAIM, Burlington, Vt.
WOLLENMAN, O. J., JR., McKinney, Tex.
WOLLENWEBER, HENRY L., Baltimore, Md.
WOOD, DAVID A., San Francisco, Calif.
WOOD, FRANCIS C., New York, N. Y.
WOOD, HAROLD, Phoenix, Ariz.
WOODRUFF, CHARLES E., Northville, Mich.
WOOLNER, LEWIS B., Rochester, Minn.
WORK, JOHN L., Montclaire, N. J.
WOZENCRAFT, JEAN PAUL, New York, N. Y.
WRIGHT, ANGUS, Los Angeles, Calif.
WRIGHT, ARTHUR W., Albany, N. Y.

WRIGHT, ROBERT B., Baltimore, Md.
WYATT, J. POYNER, Toronto, Canada

YAGUDA, ASHER, Newark, N. J.
YARDUMIAN, KRIKER Y., Pittsburgh, Pa.
YESNER, RAYMOND, Newington, Conn.
YOULAND, WILLIAM E., New York, N. Y.
YOUMANS, IVA C., Miami, Fla.
YOUNG, ANNA MAY, Cleveland, Ohio

ZAE, FREDERICK G., New York, N.Y.
ZBINDEN, THEODORE, Toledo, Ohio
ZECKWER, ISOLDE R., Philadelphia, Pa.
ZEIDMAN, IRVING, Philadelphia, Pa.
ZEIGLER, EDWIN E., Lancaster, Pa.
ZEMAN, ERWIN D., Ogden, Utah
ZILLESSEN, FREDERICK O., Easton, Pa.
ZIMMERMAN, HARRY M., New York, N.Y.
ZISKIND, JOSEPH, New Orleans, La.
ZON, LEO, Baltimore, Md.
ZUCKERMAN, SAMUEL S., Cheyenne, Wyo.
ZUNDELL, JOSEPH L., Long Beach, Calif.

GEOGRAPHIC LISTING OF DIPLOMATES

ALABAMA

BIRMINGHAM

Baker, Roger D., Medical College of Alabama (*Pathologic anatomy*)
Casey, Albert E., 2236 Highland Ave. (*Clinical pathology and pathologic anatomy*)
Cunningham, Joseph A., Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Kracke, Roy R., Dean, Medical College of Alabama (*Clinical pathology and pathologic anatomy*)
McManus, J. F. A., Medical College of Alabama (*Pathologic anatomy*)
Posey, L. C., Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Wert, Earl B., Providence Hospital (*Pathologic anatomy*)

GADSDEN

Bush, J. D., Jr., Baptist Memorial Hospital (*Clinical pathology and pathologic anatomy*)

MOBILE

Wise, I. Milton, 58 St. Joseph St. (*Clinical pathology*)

MONTGOMERY

Lafferty, Charles R., St. Margaret's Hospital (*Clinical pathology and pathologic anatomy*
Trumper, Abraham, 228 Bell Bldg. (*Clinical pathology*)

TUSKALOOSA

Beck, James S. P., Druid City Hospital (*Clinical pathology and pathologic anatomy*)

TUSKOGEE

Quinland, W. S., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

ARIZONA

PHOENIX

Frost, Thomas T., 15 E. Monroe St. (*Clinical pathology and pathologic anatomy*)
Williams, Onie O., 2020 N. 11th Ave. (*Clinical pathology and pathologic anatomy*)
Wood, Harold, Good Samaritan Hospital (*Pathologic anatomy*)

TUCSON

Fuller, Ralph H., St. Mary's Hospital, St. Mary's Road (*Pathologic anatomy*)
Hirsh, Louis, Tucson Medical Center (*Clinical pathology and pathologic anatomy*)

ARKANSAS

FAYETTEVILLE

Hoge, Solomon F., Veterans Administration (*Clinical pathology and pathologic anatomy*)

LITTLE ROCK

Kilbury, Merlin Joe, 926 Donaghey Bldg. (*Clinical pathology and pathologic anatomy*)
Nettleship, Anderson, University of Arkansas, Medical School (*Clinical pathology and pathologic anatomy*)
Schwander, Howard, University of Arkansas Medical School (*Pathologic anatomy*)

NORTH LITTLE ROCK

Wilbur, Ernest Lloyd, Veterans Administration Hopital (*Pathologic anatomy*)

CALIFORNIA

ALHAMBRA

Anderson, Melvin W., 21 South Chapel (*Clinical pathology and pathologic anatomy*)

BAKERSFIELD

Kirshbaum, J. D., Kern General Hospital (*Pathologic anatomy*)

BERKELEY

Buck, Leonard W., University of California School of Medicine (*Pathologic anatomy*)
Fishback, Hamilton R., Herrick Hospital (*Clinical pathology and pathologic anatomy*)
Singman, David, 3000 Regent St. (*Clinical pathology and pathologic anatomy*)

BEVERLY HILLS

Melnick, Perry J., 300 S. Beverly Drive (*Clinical pathology and pathologic anatomy*)

CAMP HAAN

Rubenstein, Victor G., Station Hospital (*Clinical pathology*)

CORONA

McCandless, Carl M., U. S. Naval Hospital (*Clinical pathology and pathologic anatomy*)

FRENCH CAMP

Schneider, Harry J., San Joaquin General Hospital (*Pathologic anatomy*)

FRESNO

Bocian, J. J., 3264 Huntington (*Pathologic anatomy*)

GLENDALE

Andrews, Vernon Lee, 1825 Verdugo Vista Rd. (*Clinical pathology and pathologic anatomy*)
Brown, Albert F., 318 N. Central Ave. (*Clinical pathology and pathologic anatomy*)
Curtis, Gilbert DeWitt, 509 Hill Drive (*Clinical pathology and pathologic anatomy*)
Kimball, Theodore S., 318 N. Central Ave. (*Clinical pathology and pathologic anatomy*)

LOMA LINDA

Hoxie, Edwin G., College of Medical Evangelists (*Clinical pathology and pathologic anatomy*)
Small, Carrol S., College Medical Evangelists (*Clinical pathology and pathologic anatomy*)

LONG BEACH

Tuta, Joseph A., Seaside Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Zundell, Joseph L., U. S. Naval Hospital (*Clinical pathology and pathologic anatomy*)

LOS ANGELES

Bettin, Mona E., 727 W. Seventh St. (*Clinical pathology and pathologic anatomy*)
Bonynge, Charles W., 1930 Wilshire Blvd. (*Clinical pathology*)
Budd, John W., 1407 S. Hope St. (*Clinical pathology and pathologic anatomy*)
Butt, Edward Merchant, 1200 N. State St. (*Clinical pathology and pathologic anatomy*)
Camero, Anthony R., 657 S. Westlake Avenue (*Clinical pathology and pathologic anatomy*)
Dale, Charles L., 304 N. Boyle Ave. (*Clinical pathology and pathologic anatomy*)
Edmeades, Donald T., 201 S. Beloit Ave. (*Clinical pathology and pathologic anatomy*)
Edmondson, Hugh A., 1200 N. State St. (*Pathologic anatomy*)
Fisher, H. Russel, Moore-White Clinic, 571 S. Bonnie Brae (*Clinical pathology and pathologic anatomy*)
Friedman, Nathan B., Cedars of Lebanon Hospital (*Pathologic anatomy*)
Goldblatt, Harry, Cedars of Lebanon Hospital, 4833 Fountain Ave. (*Clinical pathology and pathologic anatomy*)
Hall, Ernest M., University of S. California Medical School (*Clinical pathology and pathologic anatomy*)
Hammack, Roy W., 657 S. Westlake Ave. (*Clinical pathology and pathology anatomy*)
Hirst, Albert E., Jr., Los Angeles County General Hospital (*Pathologic anatomy*)
Huntington, Robert W., Jr., 1200 N. State St. (*Clinical pathology and pathologic anatomy*)
Hyland C. M., 4545 Sunset Blvd. (*Clinical pathology and pathologic anatomy*)

Kahler, James E., 2131 W. Third St. (*Clinical pathology and pathologic anatomy*)
Kaplan, Leo, Veterans Administration Center, Residence #285 (*Clinical pathology and pathologic anatomy*)
Knutti, Ralph E., 4614 Sunset Blvd. (*Pathologic anatomy*)
Maner, George D., 657 S. Westlake Ave. (*Clinical pathology and pathologic anatomy*)
Osborne, Roland H., 312 N. Boyle Ave. (*Clinical pathology and pathologic anatomy*)
Palmer, J. J., Veterans Administration Center, Wilshire & Sawtelle Blvds. (*Pathologic anatomy*)
Pratt, Orlyn B., 312 N. Boyle Ave. (*Clinical pathology and pathologic anatomy*)
Sommer, Melvin L., 1207 Third Ave. (*Clinical pathology and pathologic anatomy*)
Straus, Reuben, 4833 Fountain Ave. (*Clinical pathology and pathologic anatomy*)
Stuurman, Sheldon H., 3875 Wilshire Blvd. (*Pathologic anatomy*)
Tragerman, L. J., 657 S. Westlake Ave. (*Clinical pathology and pathologic anatomy*)
Wright, Angus, 657 S. Westlake Ave. (*Pathologic anatomy*)

OAKLAND

Baker, Charles F., Samuel Merritt Hospital (*Pathologic anatomy*)
Fishken, B. G., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Gerstl, Bruno, Veterans Administration Hospital (*Pathologic anatomy*)
Glenn, Robert A., Samuel Merritt Hospital (*Clinical pathology and pathologic anatomy*)
Herrmann, Walter W., Veterans Administration Hospital (*Pathologic anatomy*)
Michael, Paul, 434 30th St. (*Clinical pathology and pathologic anatomy*)
Moore, Gertrude, 353 30th St. (*Clinical pathology and pathologic anatomy*)
Oldt, Mary Ruth, Mills College (*Clinical pathology and pathologic anatomy*)
Parsons, Robert J., 2701 14th Ave. (*Pathologic anatomy*)
Shaver, John S., U. S. Naval Hospital (*Clinical pathology and pathologic anatomy*)
Walker, George A., %F. E. Davis, 5900 Ovoac Ave. (*Pathologic anatomy*)

OLIVE VIEW

Bogen, Emil, Olive View Sanatorium (*Clinical pathology and pathologic anatomy*)

PACIFIC GROVE

Simard, Ernest E., 482 Pine Ave. (*Clinical pathology and pathologic anatomy*)

PALM SPRINGS

Marks, Sidney, 710 Paseo de Anza (*Pathologic anatomy*)

PALO ALTO

Hunt, Homer H., Palo Alto Hospital (*Pathologic anatomy*)
Ragle, Harold E., 2461 Bryant St. (*Clinical pathology and pathologic anatomy*)

PALOS VERDES ESTATES

Shackford, Bartlett C., 744 Via del Monte (*Clinical pathology and pathologic anatomy*)

PASADENA

Foord, Alvin G., Huntington Memorial Hospital (*Clinical pathology and pathologic anatomy*)

POMONA

Case, Lucius W., Pomona Valley Hospital (*Clinical pathology and pathologic anatomy*)

RIVERSIDE

Baisinger, Cecil F., Community Hospital (*Clinical pathology and pathologic anatomy*)

SACRAMENTO

Blumenfeld, Charles, 1433 41st St. (*Pathologic anatomy*)
Guttman, Paul Henry, 1127 11th St. (*Clinical pathology and pathologic anatomy*)

SAN ANSELMO

Bostick, W. L., 100 Meadowcroft (*Clinical pathology and pathologic anatomy*)

SAN BERNARDINO

Benjamin, Eustace L., 780 E. Gilbert St. (*Clinical pathology and pathologic anatomy*

SAN DIEGO

Ball, Howard A., 233 A Street (*Clinical pathology and pathologic anatomy*)
DeSanto, Dominic A., Mercy Hospital, Hillcrest Dr. (*Pathologic anatomy*)
Eaton, A. W., Capt., M. C., U. S. Naval Hospital (*Clinical pathology and pathologic anatomy*)
Hartley, George, Jr., 1929 Titus St. (*Clinical pathology and pathologic anatomy*)
Pickard, Rawson J., 520 E. Street, Watts Bldg. (*Clinical pathology and pathologic anatomy*)
Sumerlin, Harold S., 2001 Fourth Ave. (*Clinical pathology and pathologic anatomy*)

SAN FRANCISCO

Aronson, Roland S., 406th Medical General Laboratory, APO 500, % Postmaster (*Pathologic anatomy*)
Biskind, Gerson R., 240 Stockton St. (*Clinical pathology and pathologic anatomy*)
Bolin, Sera E., 490 Post St. (*Clinical pathology and pathologic anatomy*)
Buck, L. W., University of California Medical School (*Pathologic anatomy*)
Carr, Jesse L., 384 Post St. (*Clinical pathology and pathologic anatomy*)
Cox, Alvin J., 2398 Sacramento St. (*Pathologic anatomy*)
Crahan, Thomas Francis, U. S. Marine Hospital (*Clinical pathology and pathologic anatomy*)
Cull, Virginia Louise, 791 27th St. (*Clinical pathology and pathologic anatomy*)
Cummins, William T., 1400 Fell St. (*Clinical pathology and pathologic anatomy*)
Dorgeloh, Justin R., St. Luke's Hospital, Twenty-Seventh and Valencia Sts. (*Clinical pathology and pathologic anatomy*)
Eichorn, Karl B., 900 Hyde St. (*Clinical pathology and pathologic anatomy*)
Friedman, Melvin, University of California Medical School (*Pathologic anatomy*)
Hawthorne, John J., 516 Sutter St. (*Clinical pathology and pathologic anatomy*)
Lake, G. C., 1215 26th Ave. (*Clinical pathology and pathologic anatomy*)
Lindsay, Stuart, University of California Medical School (*Clinical pathology and pathologic anatomy*)
Lowenhaupt, Elizabeth, University of California Medical School (*Clinical pathology and pathologic anatomy*)
Moody, Adelbert M., 2200 Hayes St. (*Clinical pathology and pathologic anatomy*)
Moon, Henry D., University of California Medical School (*Clinical pathology and pathologic anatomy*)
Rinehard, James F., University of California Medical School (*Clinical pathology and pathologic anatomy*)
Rudo, Nathan, 1330 Fifth Ave. (*Clinical pathology and pathologic anatomy*)
Satterlee, Richard Covert, 267 28th Ave. (*Clinical pathology and pathologic anatomy*)
Shoemaker, Rosemary, St. Vincent's Hospital, 2131 W. Third St. (*Clinical pathology and pathologic anatomy*)
Smith, Pearl M., 3700 California Ave. (*Clinical pathology and pathologic anatomy*)
Tessmer, Carl, Lt. Col., Director, Atomic Bomb Casualty Commission, APO 500, % Postmaster (*Clinical pathology and pathologic anatomy*)
Watson, George A., 4131 Geary Blvd. (*Clinical pathology and pathologic anatomy*)
Wood, David A., 54 Commonwealth Ave. (*Clinical pathology and pathologic anatomy*)

SAN JOSE

Dennis, Robert L., San Jose Hospital (*Pathologic anatomy*)
Duval, Charles W., San Jose Hospital (*Clinical pathology and pathologic anatomy*)
McCurdy, Thomas, O'Connor Hospital (*Clinical pathology and pathologic anatomy*)
Proescher, Frederick, Santa Clara County Hospital (*Clinical pathology and pathologic anatomy*)

SAN RAFAEL

Bostick, Warren L., San Rafael General Hospital, Marin County (*Clinical pathology and pathologic anatomy*)

SANTA ANA

Rice, Maurice M., 1516 N. Broadway (*Pathologic anatomy*)

SANTA MONICA

Hummer, George J., St. John's Hospital, 1328 22nd St. (*Pathologic anatomy*)
Kosky, Alfred A., 1250 16th St. (*Clinical pathology and pathologic anatomy*)

STANFORD

Schultz, E. W., 743 Cooksey Lane (*Clinical pathology and pathologic anatomy*)

VAN NUYS

Konwaler, Benjamin E., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Reingold, Irving M., Veterans Administration Hospital (*Pathologic anatomy*)

COLORADO

AURORA

Carson, Paul Clark, 9701 E. Calfox Ave. (*Clinical pathology*)

COLORADO SPRINGS

Geever, Erving F., 1525 Alamo Ave. (*Pathologic anatomy*)
Ryder, Charles T, 1626 Wood Ave. (*Clinical pathology and pathologic anatomy*)

DENVER

Baird, Elwood Erwin, University of Colorado School of Medicine (*Clinical pathology*)
Benner, Miriam C., 254 Metropolitan Bldg. (*Pathologic anatomy*)
Black, William C., 1933 Pearl St. (*Clinical pathology and pathologic anatomy*)
Corper, Harry J., 3800 E. Colfax (*Clinical pathology and pathologic anatomy*)
Dobos, Emeric I., 1818 Humboldt St. (*Clinical pathology and pathologic anatomy*)
Freshman, Alexander W., 234 Metropolitan Bldg. (*Pathologic anatomy*)
Kingry, Charles B., 305 Republic Bldg. (*Clinical pathology and pathologic anatomy*)
Kurland, Stanley K., 227 16th St. (*Pathologic anatomy*)
Lapi, Angelo, Office of Medical Examiner (*Pathologic anatomy*)
Lubchenco, Alexis E., Presbyterian Hospital (*Pathologic anatomy*)
McNaught, James B., University of Colorado School of Medicine (*Clinical pathology and pathologic anatomy*)
Mahon, Hugh W., Fitzsimons General Hospital (*Pathologic anatomy*)
Mills, Frances M., Denver General Hospital (*Pathologic anatomy*)
Mossberger, Joseph I., University of Colorado School of Medicine (*Clinical pathology and pathologic anatomy*)
Mugrage, Edward R., 4200 E. Ninth Ave. (*Clinical pathology and pathologic anatomy*)
Mulligan, Richard, 4200 E. Ninth Ave. (*Pathologic anatomy*)
Neubuerger, Karl T., 4200 E. Ninth St. (*Pathologic anatomy*)
Ogura, George I., Denver General Hospital (*Pathologic anatomy*)
Palmer, H. D., 1056 E. 19th Ave. (*Clinical pathology and pathologic anatomy*)
Rutledge, Enid K., 953 Garfield St. (*Pathologic anatomy*)
Steer, Arthur, Fitzsimons General Hospital (*Pathologic anatomy*)
Valentine, Eleanor H., Denver General Hospital (*Clinical pathology and pathologic anatomy*)

PUEBLO

Brown, W. O., 901 Security (*Clinical pathology and pathologic anatomy*)
Gallavan, Ella Mae, Colorado State Hospital (*Clinical pathology and pathologic anatomy*)
Maynard, Carl Wesley, 702 N Main St. (*Clinical pathology and pathologic anatomy*)

CONNECTICUT

BRIDGEPORT

Akerson, Irving B., Bridgeport Hospital (*Clinical pathology and pathologic anatomy*)
McQueeney, Andrew, 94 Elmwood Place (*Clinical pathology*)

FAIRFIELD

Fulstow, Marjorie, 570 Post Road (*Clinical pathology and pathologic anatomy*)

GREENWICH

Larimore, Louise, 100 Lake Ave. (*Clinical pathology and pathologic anatomy*)
Morris, Joyce S., Greenwich Hospital Association (*Clinical pathology and pathologic anatomy*)

HARTFORD

Hastings, Louis P., 114 Woodland St. (*Clinical pathology and pathologic anatomy*)
Hough, Perry T., 179 Beacon St. (*Clinical pathology and pathologic anatomy*)
Kendall, Ralph E., Hartford Hospital (*Clinical pathology and pathologic anatomy*)
Tennant, Robert, Hartford Hospital (*Pathologic anatomy*)

MIDDLETOWN

Fisher, Jessie Weston, 28 Crescent St. (*Pathologic anatomy*)
Katzenstein, Rolf, Middlesex Hospital (*Pathologic anatomy*)
McLeod, Christie E., 28 Crescent St. (*Clinical pathology and pathologic anatomy*)

NEW BRITAIN

Rosahn, Paul, 92 Grand Ave. (*Clinical pathology and pathologic anatomy*)

NEW HAVEN

Bartlett, Charles J. B., 183 Bishop St. (*Clinical pathology and pathologic anatomy*)
Bunting, Henry, 310 Cedar St. (*Pathologic anatomy*)
Liebow, Averill, 310 Cedar St. (*Pathologic anatomy*)
Nesbit, Robert Raymond, Hospital of St. Raphael (*Clinical pathology and pathologic anatomy*)

NEWINGTON

Flashman, David H., Veterans Administration (*Clinical pathology and pathologic anatomy*)
Yesner, Raymond, 555 Willard Ave. (*Pathologic anatomy*)

NORWALK

Barnett, Roy N., Norwalk General Hospital (*Clinical pathology and pathologic anatomy*)

NORWICH

Moore, Maurice R., 88 Central Ave. (*Clinical pathology and pathologic anatomy*)

RIDGEFIELD

Fox, Robert A. (*Pathologic anatomy*)

STAMFORD

Snavely, John Geoffrey, Stamford Hospital (*Clinical pathology and pathologic anatomy*)

TORRINGTON

Opper, Lincoln, Charlotte Hungerford Hospital (*Clinical pathology and pathologic anatomy*)

WATERBURY

Collins, Joseph O., 64 Robbins St. (*Clinical pathology and pathologic anatomy*)
Cox, Marcus E., St. Mary's Hospital (*Pathologic anatomy*)

DELAWARE

ARDEN

Pollack, Otokar Jaroslav (*Clinical pathology*)

WILMINGTON

Gay, Douglas, Eighth and Clayton St. (*Clinical pathology and pathologic anatomy*)
Hooker, John W., Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Howard, John W., 14th and Washington (*Clinical pathology and pathologic anatomy*)

DISTRICT OF COLUMBIA

WASHINGTON

Aronstein, Charles G., 1707 Columbia Road, N.W. (*Clinical pathology and pathologic anatomy*)
Ash, James Earle, Army Medical Museum (*Clinical pathology and pathologic anatomy*)
Brown, Omar Jesse, U. S. Naval Medical School (*Clinical pathology*)
Cajigas, Tomas, 1801 Eye St., N.W. (*Clinical pathology and pathologic anatomy*)
Callender, George R., 3245 Cleveland Ave., N.W. (*Clinical pathology and pathologic anatomy*)
Choisser, Roger M., 1335 H. Street, N.W. (*Clinical pathology and pathologic anatomy*)
Cornell, Virgil H., Walter Reed Hospital (*Clinical pathology and pathologic anatomy*)
Dardinski, Vincent J., Georgetown Hospital (*Pathologic anatomy*)
Dart, Raymond O., Army Institute of Pathology (*Clinical pathology and pathologic anatomy*)
Dixon, Harold M., 3456 Newark N.W. (*Clinical pathology and pathologic anatomy*)
Ernst, K. F., Army Institute of Pathology (*Clinical pathology and pathologic anatomy*)
Falisi, J. Vincent, 1507 Foxhall Road (*Clinical pathology and pathologic anatomy*)
Gore, Ira, Army Institute of Pathology (*Clinical pathology and pathologic anatomy*)
Grady, Hugh, Army Institute of Pathology (*Pathologic anatomy*)

Green, Philip P., Army Medical Museum (*Clinical pathology and pathologic anatomy*)
Haymaker, Webb, Army Institute of Pathology (*Neuropathology*)
Helwig, Elson B., Army Institute of Pathology (*Clinical pathology and pathologic anatomy*)
Holt, Rufus L., Professional Service Schools (*Clinical pathology*)
Hunter, Oscar B., Suite 1000, 915 19th St., N.W. (*Clinical pathology and pathologic anatomy*)
Hunter, Oscar B., Jr., Suite 1000, 915 19th St., N.W. (*Clinical pathology and pathologic anatomy*)
Johnson, Lent Clifton, Jr., Army Institute of Pathology (*Pathologic anatomy*)
Jones, Hugh W., Walter Reed General Hospital (*Clinical pathology and pathologic anatomy*)
Kelso, Richard E., 1834 Eye St., N.W. (*Clinical pathology*)
Klinck, Gustavus H., Jr., Army Institute of Pathology (*Clinical pathology and pathologic anatomy*)
Leffler, Harrison H., Providence Hospital (*Clinical pathology and pathologic anatomy*)
Lindsay, Janvier W., 1150 Connecticut Ave., N.W. (*Clinical pathology and pathologic anatomy*)
Monaco, A. Ralph, Gallinger Municipal Hospital (*Clinical pathology*)
Mostofi, F. K., Army Institute of Pathology (*Pathologic anatomy*)
Nelson, Arthur A., Food & Drug Administration (*Clinical pathology and pathologic anatomy*)
Neuman, Lester, 1835 Eye St., N.W. (*Clinical pathology and pathologic anatomy*)
Perry, Isabella H., Army Institute of Pathology (*Pathologic anatomy*)
Perry, Thomas M., 1335 H Street, N.W. (*Clinical pathology and pathologic anatomy*)
Rappaport, Henry, 2650 Wisconsin Ave., N.W. (*Clinical pathology and pathologic anatomy*)
Rice, Eugene C., 1150 Connecticut Ave., N.W. (*Clinical pathology and pathologic anatomy*)
Smetana, Hans F., Army Institute of Pathology (*Pathologic anatomy*)
Thompson, R. M., Army Medical Museum (*Clinical pathology and pathologic anatomy*)
Tigertt, William D., % Army Dept. (*Clinical pathology*)
Vorder Bruegge, Colin F., Army Medical Center (*Basic Science Course*) (*Pathologic anatomy*)
Walker, James W., 5000 Newport Ave. (*Pathologic anatomy*)
Whitmore, Eugene R., 2139 Wyoming Ave., N.W. (*Clinical pathology and pathologic anatomy*)
Wildman, Otis, 4700 Connecticut Ave., N. W. (*Clinical pathology and pathologic anatomy*)
Winship, Theodore, Garfield Municipal Hospital, 10th and Florida Ave. (*Pathologic anatomy*)

FLORIDA

CHATTAHOCHEE

Ruediger, Ernest H., Florida State Hospital (*Clinical pathology and pathologic anatomy*)

DAYTONA BEACH

Lippincott, L. S., Halifax District Hospital (*Clinical pathology and pathologic anatomy*)

JACKSONVILLE

Dyrenforth, Lucien Y., 1022 Park St. (*Clinical pathology and pathologic anatomy*)
Murray, Nelson A., St. Vincent's Hospital (*Clinical pathology and pathologic anatomy*)
Royce, Clayton, 1022 Park St. (*Clinical pathology and pathologic anatomy*)

MIAMI

Rockman, Jacob, P.O. Box 1971 (*Clinical pathology and pathologic anatomy*)

MIAMI BEACH

Poppiti, Robert J., St. Francis Hospital (*Pathologic anatomy*)
Sayet, Maxwell M., 420 Lincoln Road (*Pathologic anatomy*)
Stowe, W. Parker, St. Francis Hospital (*Clinical pathology*)
Wiener, Morris F., Veterans Administration Nautilus (*Pathologic anatomy*)
Youman, Iva Catherine, 653 S. W. Second St. (*Clinical pathology and pathologic anatomy*)

ORLANDO

Kerr, George R., Orange General Hospital (*Clinical pathology and pathologic anatomy*)
McJunkin, Frank A., 1015 Lancaster Dr. (*Clinical pathology and pathologic anatomy*)

PENSACOLA

Squires, Gretchen V., 304 Brent Bldg. (*Clinical pathology and pathologic anatomy*)

ST. PETERSBURG

Evans, Ira C., 516 Empire Bldg. (*Pathologic anatomy*)

TAMPA

Mills, Herbert R., 911 Citizen's Bldg. (*Clinical pathology and pathologic anatomy*)
Patterson, James N., 911 Citizen's Bldg. (*Clinical pathology and pathologic anatomy*)

WEST PALM BEACH

Johnson, V. Marklin, 513 Harvey Bldg. (*Clinical pathology and pathologic anatomy*)

GEORGIA

ATHENS

Mullins, DeWitt F., Jr., St. Mary's Hospital (*Pathologic anatomy*)

ATLANTA

Ayer, Guy D., Jr., Crawford W. Long Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Ayers, Amos J., 111 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Bishop, Everett L., 204 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Blumberg, Alfred, Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Blumberg, Joe M., Oliver General Hospital (*Clinical pathology and pathologic anatomy*)
Funke, John, 712 Durant Pl. (*Clinical pathology and pathologic anatomy*)
Klugh, George F., 139 Forrest Ave., N.E. (*Clinical pathology and pathologic anatomy*)
Madden, Sidney C., 36 Butler St., S.E. (*Pathologic anatomy*)
Matthews, Warren B., Piedmont Hospital (*Clinical pathology and pathologic anatomy*)
Norris, Jack C., 478 Peachtree St. (*Clinical pathology and pathologic anatomy*)
Sheldon, Walter H., 36 Butler St., S.E. (*Pathologic anatomy*)

AUGUSTA

Dick, Fred, Jr., University of Georgia (*Pathologic anatomy*)
Odessky, Louis, Veterans Administration Hospital (*Pathologic anatomy*)
Pund, Edgar R., University of Georgia School of Medicine (*Pathologic anatomy*)
Shepeard, Walter L., University of Georgia (*Clinical pathology and pathologic anatomy*)

CHAMBLEE

Foster, Lee N., Lawson General Veterans Administration Hospital (*Pathologic anatomy*)

COLUMBUS

Blumstein, Charles L., Columbus City Hospital (*Clinical pathology and pathologic anatomy*)
Venable, Douglas R., City Hospital (*Clinical pathology and pathologic anatomy*)

MILLEDGEVILLE

Fernan-Nunez, Marcos, Georgia State Hospital (*Clinical pathology and pathologic anatomy*)

THOMASVILLE

Saye, Ernest B., John D. Archbold Memorial Hospital (*Clinical pathology and pathologic anatomy*)

VALDOSTA

Ketchum, C. W., 1306 W. Patterson (*Clinical pathology and pathologic anatomy*)

IDAHO

BOISE

Beeman, Joseph, 310 N. First St. (*Clinical pathology and pathologic anatomy*)
Craig, Helen F., Hotel Boise (*Clinical pathology and pathologic anatomy*)

TWIN FALLS

Creed, J Woodson, Twin Falls County General Hospital (*Clinical pathology and pathologic anatomy*)

ILLINOIS

ALTON

Halley, Henry H., Jr., St. Joseph's Hospital (*Pathologic anatomy*)

BLOOMINGTON

Markowitz, Benjamin, 219 N. Main St. (*Clinical pathology*)

CHAMPAIGN

Appel, Max, Burnham City Hospital (*Pathologic anatomy*)

CHICAGO

Anday, George J., 104 S. Michigan Ave. (*Clinical pathology*)
Arcellana, Juan A., 21 West Elm Street (*Pathologic anatomy*)
Barnes, Marian, 55 East Washington St. (*Clinical pathology*)
Bennett, Granville A., 1835 W. Polk St. (*Pathologic anatomy*)
Branch, Charles F., 40 E. Erie St. (*Clinical pathology and pathologic anatomy*)
Cannon, Paul R., University of Chicago (*Clinical pathology and pathologic anatomy*)
Chapman, Bernard M., 185 N. Wabash Ave. (*Pathologic anatomy*)
Conti, Horace, 4141 N. Clarendon Ave. (*Clinical pathology and pathologic anatomy*)
Corrigan, Marion C., Michael Reese Hospital (*Pathologic anatomy*)
Croll, Diane, University Hospital, Congress St., Ogden & Wolcott Aves. (*Clinical pathology and pathologic anatomy*)
Cullison, Charles W., 1853 W. Polk St. (*Pathologic anatomy*)
Davidsohn, Israel, 2750 W. 15th Pl. (*Clinical pathology and pathologic anatomy*)
Eisensteadt, Werner F., 116 S. Michigan Ave. (*Clinical pathology*)
Goldenburg, Morris, 9948 South Claremont Ave. (*Clinical pathology and pathologic anatomy*)
Gomori, George, 950 E. 59th St (*Pathologic anatomy*)
Grams, Leslie R., Englewood Hospital (*Clinical pathology and pathologic anatomy*)
Grimm, Harold A., 551 Grant Pl. (*Clinical pathology and pathologic anatomy*)
Hagerty, C. S., St. Bernard's Hospital (*Pathologic anatomy*)
Hepler, Opal, Northwestern University School of Medicine (*Clinical pathology*)
Hirsch, Edwin F., St. Luke's Hospital (*Clinical pathology and pathologic anatomy*)
Hospers, Cornelius A., 6001 S. Green St. (*Pathologic anatomy*)
Howell, Katherine M., 6830 S. Merrill Ave. (*Clinical pathology and pathologic anatomy*)
Humphreys, Eleanor M., University of Chicago (*Pathologic anatomy*)
Josephy, Herman, 6500 Irving Park (*Pathologic anatomy*)
Kearns, Jerry Joseph, 1431 N. Claremont (*Clinical pathology and pathologic anatomy*)
King, L. S., 4204 N. Greenview Ave. (*Pathologic anatomy*)
Krakower, C. A., 1853 W. Polk St. (*Pathologic anatomy*)
Laemle, Robert Mayer, Michael Reece Hospital (*Pathologic anatomy*)
Laipply, Thomas C., Wesley Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Learner, Aaron, Norwegian American Hospital (*Clinical pathology and pathologic anatomy*)
Lederer, Arthur, 208 S. La Salle St. (*Clinical pathology and pathologic anatomy*)
Leroy, Elie P., 29th St. & Ellis Ave. (*Pathologic anatomy*)
Levine, Victor, 8046 Crandon Ave. (*Clinical pathology and pathologic anatomy*)
Levinson, Samuel A., 808 S. Wood St. (*Clinical pathology and pathologic anatomy*)
Lewis, Julian E., University of Chicago (*Clinical pathology and pathologic anatomy*)
Loeffler, Ernst, 3333 Washington St. (*Pathologic anatomy*)
Loeffler, Louis, Mercy Hospital (*Clinical pathology and pathologic anatomy*)
Mason, Coye C., 551 W. Grant Pl. (*Pathologic anatomy*)
Mavrelis, William P., Cook County Hospital (*Clinical pathology and pathologic anatomy*)
Milles, George, Augustana Hospital (*Clinical pathology and pathologic anatomy*)
Moore, J. J., 55 E. Washington St. (*Clinical pathology and pathologic anatomy*)
Nedzel, Alexander J., 1853 Polk St., (*Pathologic anatomy*)
Neiman, Benjamin, 6 N. Michigan Ave. (*Pathologic anatomy*)
Nowakovsky, Sophie T., 1512 N. Dearborn St. (*Clinical pathology*)
Parmacek, L., 55 E. Washington St. (*Clinical pathology and pathologic anatomy*)
Pilot, Isadore, Lutheran Deaconess Hospital (*Clinical pathology and pathologic anatomy*)
Pilot, Jean, 10218 S. Aberdeen St. (*Clinical pathology and pathologic anatomy*)
Pirani, Conrad C., Army Medical Laboratory, 1849 W. Pershing Road (*Pathologic anatomy*)
Popper, Hans, Cook County Hospital (*Clinical pathology and pathologic anatomy*)
Potter, Edith L., Chicago Lying-in-Hospital (*Pathologic anatomy*)
Pribram, Ernest A., Loyola University, 706 S. Lincoln St. (*Clinical pathology and pathologic anatomy*)
Ragins, Alex B., Cook County Hospital (*Clinical pathology and pathologic anatomy*)
Rosenthal, Sol R., 1853 Polk St. (*Clinical pathology and pathologic anatomy*)
Rukstinat, George J., 8001 S. Laflin St. (*Clinical pathology and pathologic anatomy*)
Saphir, Otto, Michael Reese Hospital (*Clinical pathology and pathologic anatomy*)
Schmitt, Paul G. F., 4507 N. Richmond St. (*Clinical pathology and pathologic anatomy*)
Sheehan, John F., 2520 Calumet Ave. (*Clinical pathology and pathologic anatomy*)
Simonds, James P., 234 E. Pearson St. (*Clinical pathology and pathologic anatomy*)

Steiner, Paul E., University of Chicago (*Pathologic anatomy*)
Stern, Kurt, 2750 W. 15th Place (*Pathologic anatomy*)
Stone, Fred D., 29th & Ellis Ave. (*Pathologic anatomy*)
Sweany, Henry C., 5601 N. Pulaski Rd. (*Clinical pathology and pathologic anatomy*)
Szanto, Paul B., 905 Lakeside Place (*Clinical pathology and pathologic anatomy*)
Tarlow, Lillian S., 2520 S. Calumet Ave. (*Pathologic anatomy*)
Thurston, Eric W., 242 East Walton Place (*Clinical pathology and pathologic anatomy*)
Volk, Bruno W., 1825 W. Harrison St. (*Pathologic anatomy*)
Wartman, William B., 303 E. Chicago Ave. (*Pathologic anatomy*)
Wheelock, Mark C., Passavant Hospital, 303 E. Superior St. (*Clinical pathology and pathologic anatomy*)

DOWNEY

Jacobson, Moses A., Veterans Administration (*Clinical pathology*)

ELGIN

Olson, Ernest S., Sherman Hospital (*Pathologic anatomy*)

EVANSTON

Henry, James W., St. Francis Hospital (*Clinical pathology and pathologic anatomy*)
McCarter, John C. 2650 Ridge Ave., (*Clinical pathology and pathologic anatomy*)
Schiller, Walter, 1401 Lake St. (*Pathologic anatomy*)
Skinsnes, Olaf K., 2609 Ridge Ave. (*Pathologic anatomy*)

HERRIN

Bornstein, Frederick, Herrin Hospital (*Pathologic anatomy*)

HIMSDALE

Benichek-Langford, Maria, Himsdale (*Pathologic anatomy and clinical pathology*)

HIGHLAND PARK

Wilson, Hugh, 957 N. St. Johns Pl. (*Pathologic anatomy*)

HINES

Costich, Kenneth J., Veterans Administration Hospital (*Pathologic anatomy*)
McNamara, William L., Veterans Administration Hospital (*Pathologic anatomy*)
Schrek, Robert, Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

JACKSONVILLE

McKinley, Hugh A., Box 382 (*Clinical pathology and pathologic anatomy*)

OAK PARK

Hill, Lewis R., 1011 Lake St. (*Clinical pathology and pathologic anatomy*)
Kiselis, John P., 518 North Austin Blvd. (*Clinical pathology*)

PEORIA

Kraus, John E., 318 Biltmore (*Clinical pathology and pathologic anatomy*)
Manshardt, Donald O., Methodist Hospital of Central Illinois (*Pathologic anatomy*)

QUINCY

Cohen, Frank, Illinois National Bank Bldg. (*Clinical pathology and pathologic anatomy*)

ROCKFORD

Alexander, Marshall O., 507 Chestnut (*Pathologic anatomy*)
Kramer, G. B., Swedish American Hospital (*Clinical pathology and pathologic anatomy*)
Matthews, Arthur R. K., 1401 E. State St. (*Clinical pathology and pathologic anatomy*)

SPRINGFIELD

Steen, Harry M., St. John's Hospital (*Clinical pathology and pathologic anatomy*)
Vass, Aloysius, Memorial Hospital (*Clinical pathology and pathologic anatomy*)

URBANA

Lev, Maurice, Medical College of Illinois (*Clinical pathology and pathologic anatomy*)
McClure, George Y., 602 W. University Ave. (*Pathologic anatomy*)

WILMETTE

Brown, H. Ivan, 1119 Lake Avenue (*Clinical pathology and pathologic anatomy*)

INDIANA

COLUMBUS

Adler, David L., Bartholomew County Hospital (*Clinical pathology and pathologic anatomy*)

EVANSVILLE

Bennett, A. P., Welborn Memorial Baptist Hospital (*Clinical pathology and pathologic anatomy*)
Mills, Fred E., Protestant Deaconess Hospital (*Pathologic anatomy*)
Ratcliffe, Albert W., 510 S. E. First St. (*Clinical pathology and pathologic anatomy*)

FORT WAYNE

Rabson, S. M., 2440 S. Fairfield (*Pathologic anatomy*)
Rhamy, Bonnelle W., 347 W. Berry St. (*Clinical pathology and pathologic anatomy*)
Schlademan, Karl R., 347 W. Berry St. (*Clinical pathology and pathologic anatomy*)
Schneider, Louis A., 4621 South Park Drive (*Clinical pathology and pathologic anatomy*)
Tomlinson, Wray J., 347 W. Berry St. (*Clinical pathology and pathologic anatomy*)

GARY

Balkin, Ruth B., Methodist Hospital (*Pathologic anatomy*)

INDIANAPOLIS

Arbogast, J. L., 1040 W. Michigan Ave. (*Clinical pathology and pathologic anatomy*)
Banks, Horace M., 1604 N. Capitol Ave., (*Clinical pathology and pathologic anatomy*)
Culbertson, Clyde G., Lilly Research Laboratory (*Clinical pathology and pathologic anatomy*)
Dublin, William B., Indianapolis City Hospital (*Clinical pathology and pathologic anatomy*)
Forry, Frank, Indiana University Med. Center (*Clinical pathology and pathologic anatomy*)
Harris, Paul N., 740 South Alabama St. (*Pathologic anatomy*)
Haymond, Joseph L., 605 East 38th St. (*Clinical pathology and pathologic anatomy*)
Hoyt, Lester H., Methodist Hospital (*Pathologic anatomy*)
Michael, Amos C., Indiana University School of Medicine (*Pathologic anatomy*)
Rice, Thurman B., Indiana University School of Medicine (*Clinical pathology*)
Rosenbaum, David, Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Shimer, Will, St. Vincent's Hospital (*Clinical pathology*)
Thornton, Harold C., St. Vincent's Hospital (*Clinical pathology and pathologic anatomy*)

LAFAYETTE

Bayley, William E., 2400 South St., (*Clinical pathology and pathologic anatomy*)
Hunter, Frank P., 300 Main St. (*Clinical pathology and pathologic anatomy*)
McFadden, James M., St. Elizabeth Hospital (*Clinical pathology and pathologic anatomy*)

MUNCIE

Cole, Russel E., P. O. Box 611 (*Clinical pathology*)
Montgomery, Lall G., Ball Memorial Hospital (*Clinical pathology and pathologic anatomy*)

SOUTH BEND

Culbertson, Carl S., 531 N. Main St. (*Clinical pathology and pathologic anatomy*)
Giordano, A. S. 531 N. Main St. (*Clinical pathology and pathologic anatomy*)

TERRE HAUTE

Blum, Leon L., 512 Tribune Bldg. (*Clinical pathology*)
Selsam, Etta B., 202 Merchant's National Bank (*Clinical pathology and pathologic anatomy*)

IOWA

CEDAR RAPIDS

Mulsow, Frederick, 511 Highley Bldg. (*Clinical pathology*)
Weland, R. E., Mercy Hospital (*Clinical pathology and pathologic anatomy*)

CLINTON

Stewart, John K., Bluff Road (*Clinical pathology and pathologic anatomy*)

DAVENPORT

Lamb, Frederick H., 220 Main St. (*Clinical pathology and pathologic anatomy*)
Pheteplace, Willard S., Mercy Hospital (*Pathologic anatomy*)

DES MOINES

Birge, R. F., 1200 Pleasant St. (*Clinical pathology and pathologic anatomy*)
Coleman, Francis C., Mercy Hospital (*Clinical pathology and pathologic anatomy*)
Cross, K. R., 1601 45th St. (*Pathologic anatomy*)

DUBUQUE

Thorness, Edwin T., St. Joseph Mercy Hospital (*Clinical pathology and pathologic anatomy*)

IOWA CITY

Boyd, Eugene J., State University of Iowa, School of Medicine (*Pathologic anatomy*)
Carter, John Robert, State University of Iowa, School of Medicine (*Pathologic anatomy*)
Warner, Emory D., 133 Medical Laboratory Bldg. (*Clinical pathology and pathologic anatomy*)

MOUNT PLEASANT

Oesterlin, Ernst J., State Hospital (*Clinical pathology and pathologic anatomy*)

SIOUX CITY

Starry, Allen C., 2319 Douglas St. (*Clinical pathology and pathologic anatomy*)

KANSAS

KANSAS CITY

Hamilton, Tom R., Providence Hospital (*Pathologic anatomy*)
Summerville, Ward W., Bethany Methodist Hospital (*Pathologic anatomy*)
Stowell, Robert E., Medical Center, University of Kansas (*Pathologic anatomy*)
Wahl, Harry R., 39th & Hudson Road (*Clinical pathology and pathologic anatomy*)
Weber, Clarence J., University of Kansas Medical School (*Clinical pathology*)

LAWRENCE

Sherwood, Noble P., 517 Snow Hall, U. of Kansas (*Clinical pathology*)

TOPEKA

Fink, A. A., 2504 Granthurst Ave. (*Clinical pathology and pathologic anatomy*)
Gichner, Manuel G. Veterans Administration Hospital (*Clinical pathology*)
Lattimore, John L., 618 Mills Bldg. (*Clinical pathology and pathologic anatomy*)

WICHITA

Callahan, William P., Suite 1106 Brown Bldg. (*Pathologic anatomy*)
Hellwig, Christian A., St. Francis Hospital (*Clinical pathology and pathologic anatomy*)
Murphy, Lyman C., Wichita Hospital (*Clinical pathology and pathologic anatomy*)
Stofer, Bert E., Wesley Hospital (*Pathologic anatomy*)

KENTUCKY

LEXINGTON

Maxwell, Elmer S., 190 N. Upper St. (*Clinical pathology and pathologic anatomy*)
Randall, Charles C., 149 Cherokee Park (*Clinical pathology and pathologic anatomy*)
Sweets, Henry H., Jr., 109 W. Second St. (*Clinical pathology and pathologic anatomy*

LOUISVILLE

Allen, John D., 740 Francis Bldg. (*Clinical pathology and pathologic anatomy*)
Allen, William Hogue, 740 Francis Bldg. (*Clinical pathology*)
Black, Sam H., St. Joseph Infirmary (*Pathologic anatomy*)
Comforth, Elizabeth L., Louisville General Hospital (*Pathologic anatomy*)
Gordon, Harold, Nichols Veterans Administration Hospital (*Pathologic anatomy*)
Greenwood, Eunice Sterling, Norton Infirmary (*Clinical pathology and pathologic anatomy*)
Kuhns, Dwight Moody, 2541 Dell Road, Seneca Gardens (*Clinical pathology and pathologic anatomy*)
Miller, Aura J., 101 W. Chestnut St. (*Clinical pathology and pathologic anatomy*)
Townsend, Eleanor, Kentucky Baptist Hospital (*Clinical pathology*)
Weeter, Harry M., 332 W. Broadway (*Clinical pathology and pathologic anatomy*)
Williams, John, Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

LOUISIANA

ALEXANDRIA

Cardwell, Edward S., Jr., Baptist Hospital (*Pathologic anatomy*)

BATON ROUGE

Beven, John L., Our Lady of the Lake Hospital (*Clinical pathology and pathologic anatomy*)
Colvin, Samuel H., Jr., Baton Rouge General Hospital (*Clinical pathology and pathologic anatomy*)
Heibner, Winston C., Baton Rouge General Hospital (*Pathologic anatomy*)

LAKE CHARLES

Hebert, Louis A., 813 Pujo St. (*Clinical pathology*)

MONROE

Klam, Najeeb, St. Francis Sanatorium (*Pathologic anatomy*)

NEW ORLEANS

Adams, George, Quarters 2, U. S. Marine Hospital (*Pathologic anatomy*)
Arey, James Blanding, Tulane University School of Medicine (*Pathologic anatomy*)
Bacher, Wilhelmina C., 3528 Chestnut St. (*Pathologic anatomy*)
Bass, Elizabeth, 1007 American Bank Bldg. (*Clinical pathology*)
Carrera, Guillermo, 1430 Tulane Ave. (*Pathologic anatomy*)
Connell, John H., 803 Audubon Bldg. (*Clinical pathology and pathologic anatomy*)
D'Aunoy, Joseph R., Medical Center, Louisiana State University (*Clinical pathology and pathologic anatomy*)
Dunlap, Charles E., 1430 Tulane Ave. (*Pathologic anatomy*)
Friedrichs, Andrew V., 921 Canal St. (*Clinical pathology and pathologic anatomy*)
Harris, William H., 832 Maison Blanche Bldg. (*Clinical pathology and pathologic anatomy*)
Hartwell, R. M., Hotel Dieu (*Clinical pathology and pathologic anatomy*)
Hauser, George H., 730 Audubon Bldg. (*Clinical pathology and pathologic anatomy*)
Henthorne, J. C., 1445 Joseph St. (*Clinical pathology and pathologic anatomy*)
Hertzog, Ambrose J., Touro Infirmary (*Clinical pathology and pa hologic anatomy*)
Holman, Russell L., 1542 Tulane Ave. (*Clinical pathology and pathologic anatomy*)
Lawson, Edwin H., 2700 Napoleon (*Clinical pathology and pathologic anatomy*)
Maher, Aldea, 1110 American Bank Bldg. (*Clinical pathology and pathologic anatomy*)
Moss, Emma S., Charity Hospital (*Clinical pathology and pathologic anatomy*)
Pizzolato, Philip, 2713 Dante St. (*Clinical pathology and pathologic anatomy*)
Randall, William Spears, Ochsner Clinic (*Clinical pathology*)
Seeman, W. H., 1577 Henry Clay Ave. (*Clinical pathology and pathologic anatomy*)
Sternberg, William H., 1430 Tulane Ave. (*Pathologic anatomy*)
Swan, Lawrence L., U. S. Marine Hospital (*Clinical pathology and pathologic anatomy*)
Ziskind Joseph, Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

SHREVEPORT

Butler, Willis P., P. O. Box 135 (*Clinical pathology and pathologic anatomy*)
Mathews, W. R., 1240 Texas Ave. (*Pathologic anatomy*)

MAINE

AUBURN

Goodof, Irving I., 392 Turner St. (*Clinical pathology and pathologic anatomy*)

BANGOR

Morris, Lloyd E., Eastern Maine General Hospital (*Clinical pathology and pathologic anatomy*)
Wadsworth, Richard C., Eastern Maine General Hospital (*Pathologic anatomy*)

LEWISTON

Gottlieb, Julius, Central Maine General Hospital (*Clinical pathology and pathologic anatomy*)

PORTLAND

Ferguson, Franklin F., Maine General Hospital (*Clinical pathology and pathologic anatomy*)
Porter, Joseph E., 22 Arsenal St. (*Clinical pathology and pathologic anatomy*)

MARYLAND

BALTIMORE

Beissinger, Heinz, 5201 Pleasant St. (*Pathologic anatomy*)
Binford, C. N., U. S. Marine Hospital (*Clinical pathology and pathologic anatomy*)
Carnes, William H., Johns Hopkins Hospital (*Pathologic anatomy*)
Dunn, Robert C., U. S. Public Health Service (*Pathologic anatomy*)
Follis, R. H., John Hopkins University (*Pathologic anatomy*)
Geschickter, Charles F., Lt. Comdr., 715 W. Charles St. (*Pathologic anatomy*)
Hollander, David H., 6 E. 30th St. (*Pathologic anatomy*)
Light, Frederick W., 719 E. Cold Spring Lane (*Clinical pathology and pathologic anatomy*)
Maldeis, Howard J., 4421 Marble Hall Rd., Apt. 279 (*Clinical pathology and pathologic anatomy*)
Merkel, Walter, Union Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Norwood, Vernon H., Church Home and Hospital (*Clinical pathology and pathologic anatomy*)
Reimann, D. L., University of Maryland (*Pathologic anatomy*)
Rudo, Nathan, 3523 Holmes Ave. (*Clinical pathology and pathologic anatomy*)
Sacks, Milton S., University Hospital (*Clinical pathology and pathologic anatomy*)
Spencer, Hugh R., University of Maryland School of Medicine (*Pathologic anatomy*)
Stewart, Charles W., 6 East Read Street (*Clinical pathology and pathologic anatomy*)
VandeGrift, William B., 211 Ridgemead Road (*Pathologic anatomy*)
Wagner, John A., 29 S. Green St. (*Pathologic anatomy*)
Warner, Carrol G., Baltimore City Hospital (*Pathologic anatomy*)
Weinberg, Tobias, Sinai Hospital (*Clinical pathology and pathologic anatomy*)
Wollenweber, Henry L., 225 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Wright, Robert B., University of Maryland School of Medicine (*Clinical pathology and pathologic anatomy*)
Zoen, Leo, U. S. Marine Hospital (*Clinical pathology and pathologic anatomy*)

BETHESDA

Ashburn, L. L., National Institute of Health (*Pathologic anatomy*)
Bragdon, Joseph, National Institute of Health, (*Pathologic anatomy*)
Dunn, Thelma B., National Cancer Institute (*Pathologic anatomy*)
Endicot, K. M., National Institute of Health (*Clinical pathology and pathologic anatomy*)
Hueper, Wilhelm C., 8495 Rockville Pike (*Pathologic anatomy*)
Lillie, Ralph D., National Institute of Health (*Pathologic anatomy*)
Peers, James H., National Institute of Health (*Clinical pathology and pathologic anatomy*)
Perrin, Theodore, National Institute of Health (*Pathologic anatomy*)
Silliphant, William M., U. S. N. Medical Center (*Clinical pathology and pathologic anatomy*)
Stewart, Harold I., National Institute of Health (*Clinical pathology and pathologic anatomy*)
Tullis, John L., Naval Research Institute, National Naval Medical Center (*Pathologic anatomy*)

BLADENSBURG

Manion, William C., 5353 Quincy Place (*Clinical pathology and pathologic anatomy*)

COLLEGE PARK

Kreschner, Harold W., 7406 Dickinson (*Pathologic anatomy*)

CUMBERLAND

Skitarelic, Benedict, Memorial Hospital (*Pathologic anatomy*)

FORT GENERAL GEORGE MEADE

Gilmore Hugh R., Jr., Col., 2nd Army Area Laboratory (*Clinical pathology and pathologic anatomy*)

FREDERICK

Pollack, Abou D., Camp Detrick (*Clinical pathology and pathologic anatomy*)

HAGERSTOWN

Butterfield, Paul W., Washington County Hospital (*Pathologic anatomy*)

SILVER SPRINGS

Highman, Benjamin, 8712 Colesville Road (*Pathologic anatomy*)

MASSACHUSETTS

ANDOVER

Glidden, H. Spencer, 67 Cheever Circle (*Pathologic anatomy*)

BOSTON

Bailey, Orville T., Harvard Medical School (*Pathologic anatomy*)
Belding, David L., 80 E. Concord St. (*Clinical pathology and pathologic anatomy*)
Canavan, Myrtelle M., 2061 Dorchester Ave. (*Pathologic anatomy*)
Castleman, Benjamin, Massachusetts General Hospital (*Pathologic anatomy*)
Dalrymple, Sidney C., Commandant's Office, First Naval District, Fargo Bldg., 495 Summer St. (*Clinical pathology and pathologic anatomy*)
Diamond, Israel, Children's Hospital (*Pathologic anatomy*)
Dunbar, Frank, H., 43 Bay State Rd. (*Clinical pathology*)
Eichwald, Ernst J., 300 Longwood Ave. (*Pathologic anatomy*)
Fanger, Herbert, Peter Bent Brigham Hospital (*Pathologic anatomy*)
Farber, Sidney, 300 Longwood Ave. (*Pathologic anatomy*)
Freiman, David G., Massachusetts General Hospital (*Pathologic anatomy*)
Hicks, Samuel P., New England Deaconess Hospital (*Pathologic anatomy*)
Jetter, Walter W., 25 Shattuck St. (*Clinical pathology and pathologic anatomy*)
Leary, Olga C., 43 Bay State Rd. (*Pathologic anatomy*)
MacMahon, Harold E., 416 Huntington Ave. (*Pathologic anatomy*)
Mallory, G. Kenneth, Mallory Institute of Pathology (*Clinical pathology and pathologic anatomy*)
Mallory, Tracy B., Massachusetts General Hospital (*Clinical pathology and pathologic anatomy*)
Mann, Bernard F., 195 Pilgrim Rd. (*Clinical pathology and pathologic anatomy*)
McPeak, Elsie M., 195 Pilgrim Rd. (*Clinical pathology and pathologic anatomy*)
Meissner, William A., 195 Pilgrim Road (*Clinical pathology and pathologic anatomy*)
Moritz, Alan R., 25 Shattuck St. (*Clinical pathology and pathologic anatomy*)
Olken, Harry G., 416 Hungtinton Ave. (*Pathologic anatomy*)
Parker, Frederic, Jr., 780 Massachusetts Ave. (*Clinical pathology and pathologic anatomy*)
Robbins, Stanley L., Boston City Hospital (*Pathologic anatomy*)
Rooney, J. Stewart, 53 Parker Hill Ave. (*Clinical pathology and pathologic anatomy*)
Schlesinger, Monroe J., 330 Brookline Ave. (*Clinical pathology and pathologic anatomy*)
Simmons, J. S., Harvard School of Public Health (*Clinical pathology*)
Sniffen, Ronald C., Massachusetts General Hospital (*Pathologic anatomy*)
Sprinz, Helmuth S., 416 Huntington Ave. (*Pathologic anatomy*)
Taft, Edgar B., Mallory Institute of Pathology (*Clinical pathology and pathologic anatomy*)
Warren, Shields, 195 Pilgrim Road (*Clinical pathology and pathologic anatomy*)
Wolbach, S. B., Harvard Medical School (*Pathologic anatomy*)

BRIGHTON

Shapiro, Morris W., 66 Chiswick Road (*Pathologic anatomy*)

BROOKLINE

Gates, Olive, 1397 Beacon St. (*Pathologic anatomy*)

CAMBRIDGE

Baldauf, Leon K., Cambridge City Hospital (*Pathologic anatomy*)
Schulz, Reuben Z., Harvard Medical School (*Clinical pathology and pathologic anatomy*)

EAST WEYMOUTH

Bates, Lewis B., 45 Randall Ave. (*Clinical pathology and pathologic anatomy*)

FALL RIVER

Connerty, Harold V., Union Hospital (*Clinical pathology and pathologic anatomy*)
Freeman, William, Truesdale Hospital (*Pathologic anatomy*)

FOXBORO

Flashman, David H., Foxboro State Hospital (*Clinical pathology and pathologic anatomy*)

FRAMINGHAM

Fienberg, Robert, Cushing Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

HOLYOKE

Kaufmann, William, 304 Jackson (*Pathologic anatomy*)

JAMAICA PLAIN

LeCompte, Phillip M., 1153 Centre St. (*Clinical pathology and pathologic anatomy*)

LAWRENCE

Jolliffe, Leslie S., Lawrence General Hospital (*Pathologic anatomy*)

LEXINGTON

Osgood, Rudolf, 35 Eaton Road (*Pathologic anatomy*)

LOWELL

Rodger, James Y., 22 Garden Road (*Clinical pathology and pathologic anatomy*)

MALDEN

MacKenzie, Michael V., Malden Hospital (*Pathologic anatomy*)

NEW BEDFORD

Wason, Isabel M., 14 Rotch St. (*Clinical pathology and pathologic anatomy*)

NEWTON CENTER

Houghton, J. D., 636 Dedham Street (*Pathologic anatomy*)

NEWTON HIGHLANDS

Elton, Norman W., 28 Cochituate Road (*Clinical pathology and pathologic anatomy*)

NEWTON LOWER FALLS

Skinner, David, 2014 Washington Street (*Clinical pathology and pathologic anatomy*)

NEWTONVILLE

Muller, Gulli Lindh, 56 Morse Rd. (*Clinical pathology and pathologic anatomy*)

PITTSFIELD

Scoville, Helen M., 741 North St. (*Clinical pathology and pathologic anatomy*)

ROSLINDALE

Steele, Albert E., 63 Robert St. (*Clinical pathology*)

SALEM

Keane, Geoffrey P., Salem Hospital (*Pathologic anatomy*)
Nickerson, Donald A., 81 Highland Ave. (*Clinical pathology and pathologic anatomy*)

SPRINGFIELD

Dwyer, John E., 146 Chestnut St. (*Clinical pathology*)
Jones, Fred D., 20 Maple St. (*Clinical pathology and pathologic anatomy*)
Morris, Lloyd E., Springfield Hospital (*Clinical pathology and pathologic anatomy*)

WALTHAM

Strassman, George S., Met. State Hospital (*Pathologic anatomy*)

WELLFLEET

Ehrlich, Joseph C., (*Clinical pathology and pathologic anatomy*)

WESTBOROUGH

Pierce, Lydia B., Westborough State Hospital (*Clinical pathology and pathologic anatomy*)

WEST ROXBURY

Larkin, John J., 215 Perham St. (*Clinical pathology and pathologic anatomy*)
Looney, Joseph M., Veterans Administration Hospital (*Clinical pathology*)

WEST SPRINGFIELD

Rini, Joseph, 16 Reed Street (*Pathologic anatomy*)

WORCESTER

Casale, Walter, St. Vincent Hospital (*Pathologic anatomy*)
Goodale, Raymond H., 36 Otsega Rd. (*Clinical pathology and pathologic anatomy*)

MICHIGAN

ANN ARBOR

French, A. James, University Hospital (*Pathologic anatomy*)
Hinerman, Dorin L., University of Michigan Medical School (*Pathologic anatomy*)
Wanstrom, Ruth C., University of Michigan Medical School (*Pathologic anatomy*)
Weller, Carl V., University of Michigan (*Clinical pathology and pathologic anatomy*)

BATTLE CREEK

Humphrey, Arthur A., Community Hospital Assn. (*Pathologic anatomy*)
Palmer, Jay J., Percy Jones General Hospital (*Pathologic anatomy*)
Schelm, G. W., Leila Y. Post Montgomery Hospital (*Clinical pathology and pathologic anatomy*)

DEARBORN

Linn, Herman J., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

DETROIT

Beaver, Donald C., Woman's Hospital (*Clinical pathology and pathologic anatomy*)
Berman, Lawrence, 1516 St. Antoine St. (*Pathologic anatomy*)
Brines, Osborne A., 1516 St. Antoine St. (*Clinical pathology and pathologic anatomy*)
Brosius, William L., 2349 Leslie Ave. (*Clinical pathology and pathologic anatomy*)
Christopher, James G., 1204 Kales Bldg., 76 West Adams Ave. (*Pathologic anatomy*)
Clark, Harry L., 634 Maccabees Bldg. (*Clinical pathology*)
Gardner, Lawrence W., Mt. Carmel Mercy Hospital (*Pathologic anatomy*)
Hartman, Frank W , Henry Ford Hospital (*Clinical pathology and pathologic anatomy*)
Hasley, Daniel E., 1516 St. Antoine St., (*Clinical pathology*)
Kasper, Joseph A., 1151 Taylor Ave. (*Clinical pathology and pathologic anatomy*)
Kaump, Donald H., Providence Hospital (*Clinical pathology and pathologic anatomy*)
Lossman, Robert T., Receiving Hospital (*Pathologic anatomy*)
Madsen, Martha E., 3245 E. Jefferson Ave. (*Clinical pathology and pathologic anatomy*)
Maun, Mark E., 1420 St. Antoine St. (*Pathologic anatomy*)
Morse, Plinn F., Harper Hospital (*Clinical pathology and pathologic anatomy*)
Owen, Clarence I., Grace Hospital (*Clinical pathology and pathologic anatomy*)
Stryker, Walter A., St. Mary's Hospital (*Pathologic anatomy*)

ELOISE

Gould, Sylvester E., Wayne County General Hospital (*Clinical pathology and pathologic anatomy*)

FLINT

Backus, G. R., 3131 Westwood Parkway (*Pathologic anatomy*)
Jermstad, Robert J., 623 Mott Foundation Bldg. (*Pathologic anatomy*)

GRAND RAPIDS

Ducey, E. F., St. Mary's Hospital (*Clinical pathology and pathologic anatomy*)
Payne, C. Allen, Blodgett Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Van Pernis, Paul V., Butterworth Hospital (*Pathologic anatomy*)

JACKSON

Ahronheim, J. H., W. A. Foote Memorial Hospital (*Clinical pathology and pathologic anatomy*)

KALAMAZOO

DeGroat, Albert, 406 Fairfax St. (*Clinica lpathology and pathologic anatomy*)
Prentice, Hazel R., 3404 Oakland Dr. (*Clinical pathology and pathologic anatomy*)

LANSING

Black, Charles E., St. Lawrence Hospital (*Pathologic anatomy*)

MARQUETTE

Amolsch, Arthur, St. Luke's Hospital (*Clinical pathology and pathologic anatomy*)

NORTHVILLE

Woodruff, Charles E., Maybury Sanatorium (*Pathologic anatomy*)

PONTIAC

Olsen, Richard E., St. Joseph Mercy Hospital (*Clinical pathology and pathologic anatomy*)
Harsh, Robert C., Pontiac General Hospital (*Clinical pathology and pathologic anatomy*)

SAGINAW

Bucklin, Robert van Zandt, 1447 North Harrison St. (*Clinical pathology and pathologic anatomy*)
Lohr, Oliver W., 537 Millard St. (*Clinical pathology and pathologic anatomy*)

WYANDOTTE

Mayner, Frederick A., Wyandotte General Hospital (*Clinical pathology and pathologic anatomy*)

MINNESOTA

DULUTH

Berdez, George L., St. Mary's Hospital (*Clinical pathology and pathologic anatomy*)
Knoll, William V., St. Mary's Hospital (*Pathologic anatomy*)
Wells, Arthur H., St. Luke's Hospital (*Clinical pathology and pathologic anatomy*)

MINNEAPOLIS

Bell, Elixious T., University of Minnesota (*Pathologic anatomy*)
Clawson, Benjamin J., 500 Delaware St., S. E. (*Pathologic anatomy*)
Drake, Charles R., 600 Physicians and Surgeons Bldg. (*Clinical pathology*)
Hebbel, Robert, University of Minnesota (*Pathologic anatomy*)
James, Ellery M., 1412 E. 24th St. (*Pathologic anatomy*)
Koucky, Rudolph W., 2500 S. Sixth St. (*Clinical pathology and pathologic anatomy*)
Lind, Carl J. Jr., 3104 45 Ave. S. (*Pathologic anatomy*)
Llewellyn, M. B., Asbury Hospital, 916 E. 15th St. (*Pathologic anatomy*)
Lofsness, Stanley V., Swedish Hospital (*Pathologic anatomy*)
Lott, Frederick H., Abbott Hospital (*Pathologic anatomy*)
Lufkin, Nathaniel H., 920 7th St. South (*Clinical pathology and pathologic anatomy*)
McCartney, James S., Jr., University of Minnesota (*Clinical pathology and pathologic anatomy*)

ROCHESTER

Baggenstoss, Archie H., 102 Second Ave. (*Clinical pathology and pathologic anatomy*)
Bennett, Warren A., Mayo Clinic (*Clinical pathology and pathologic anatomy*)
Broders, Albert C., Mayo Clinic (*Clinical pathology and pathologic anatomy*)
Edwards, Jesse E., Mayo Clinic (*Pathologic anatomy*)
Kernohan, James W., Mayo Clinic (*Pathologic anatomy*)
McDonald, John R., Mayo Clinic (*Clinical pathology and pathologic anatomy*)
Magath, T. B., Mayo Clinic (*Clinical pathology*)
Mann, Frank D., Mayo Clinic (*Clinical pathology*)
Parkhill, Edith M., Mayo Clinic (*Clinical pathology and pathologic anatomy*)
Pease, Gertrude L., Mayo Clinic (*Clinical pathology and pathologic anatomy*)
Sanford, Arthur H., Mayo Clinic (*Clinical pathology*)
Sayre, George P , Mayo Clinic (*Pathologic anatomy*)
Weed, Lyle A., Mayo Clinic (*Pathologic anatomy*)
Woolner, Lewis B., Mayo Clinic (*Clinical pathology and pathologic anatomy*)

ST. CLOUD

Stangl, P. E., St. Cloud Hospital (*Pathologic anatomy*)

ST. PAUL

Ikeda, Kano, 125 W. College Ave. (*Clinical pathology and pathologic anatomy*)
Kenyon, Thomas J., St. Joseph's Hospital (*Clinical pathology and pathologic anatomy*)
Kvitrud, Gilbert, 1700 University Ave. (*Clinical pathology*)
Noble, John F., Ancker Hospital (*Clinical pathology and pathologic anatomy*)

MISSISSIPPI

GREENVILLE

White, Earl T., 310 Arnold Ave. (*Clinical pathology*)

JACKSON

Bratley, Forrest Groves, Mississippi Valley Hospital (*Clinical pathology and pathologic anatomy*)
Nauman, Hans N., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

VICKSBURG

Fenstermacher, Richard H., 920 Crawford Street (*Clinical pathology and pathologic anatomy*)
Moore, Robert M., Vicksburg Clinic (*Pathologic anatomy*)

MISSOURI

COLUMBIA

Johnson, Richard, Ellis Fischel State Hospital (*Pathologic anatomy*)
Neal, M., Pinson, Missouri University School of Medicine (*Clinical pathology and pathologic anatomy*)

EXCELSIOR SPRINGS

Bailey, William H., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

JEFFERSON CITY

Adams, Charles F., Division of Health (*Clinical pathology and pathologic anatomy*)

KANSAS CITY

Allebach, Hobart K. B., Research Hospital (*Clinical pathology and pathologic anatomy*)
Buhler, Victor B., K. C. General Hospital (*Clinical pathology and pathologic anatomy*)
Helwig, Ferdinand C., St. Luke's Hospital (*Pathologic anatomy*)
Jones, Maurice L., 306 E. 12th St. (*Clinical pathology and pathologic anatomy*)
Kerr, Russell W., St. Joseph's Hospital (*Pathologic anatomy*)
Leitch, C. G., 1109 Professional Bldg. (*Clinical pathology*)
Upsher, Albert E., St. Mary's Hospital (*Clinical pathology and pathologic anatomy*)

KIRKWOOD

Blumenthal, Herman, 301 Alters Place (*Pathologic anatomy*)

ST. LOUIS

Ackerman, Lauren V., Barnes Hospital, (*Clinical pathology and pathologic anatomy*)
Allen, Henry C., 634 N. Grand Blvd. (*Clinical pathology and pathologic anatomy*)
Allen, Hollis N., 634 N. Grand Blvd. (*Clinical pathology and pathologic anatomy*)
Brown, Wilson G., St. Louis County Hospital (*Clinical pathology and pathologic anatomy*)
Fleisher, Moyer S., 216 S. Kingshighway (*Clinical pathology*)
Frerichs, John B., Barnard Hospital (*Pathologic anatomy*)
Gray, Samuel H., Jewish Hospital (*Clinical pathology and pathologic anatomy*)
Hagebusch, Omer E., 4500 Olive St. (*Clinical pathology and pathologic anatomy*)
Hall, W. E. B., 919 N. Taylor Ave. (*Clinical pathology and pathologic anatomy*)
Kannerstein, Milton, Jefferson Barracks Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Katz, Samuel D., 3903 Olive St. (*Clinical pathology and pathologic anatomy*)
Klenk, Charles L., 420 Met. Bldg. (*Clinical pathology*)
Moore, Robert A., Washington University School of Medicine (*Pathologic anatomy*)
Moragues, Vincent, 1402 South Grand Blvd. (*Pathologic anatomy*)
Peñas, Manuel D., Washington University School of Medicine (*Pathologic anatomy*)
Pinkerton, Henry, 1402 S. Grand Blvd. (*Pathologic anatomy*)
Portuondo, Bonaventure C., 3520 Chippewa St. (*Clinical pathology and pathologic anatomy*)
Roberts, John R., 3720 Washington Blvd. (*Clinical pathology and pathologic anatomy*)
Saxton, John A., 1430 Carroll St. (*Pathologic anatomy*)
Siebert, Walter J., 2415 N. Kingshighway (*Clinical pathology and pathologic anatomy*)
Thompson, Ralph L., 607 N. Grand Blvd. (*Pathologic anatomy*)

SPRINGFIELD

Hanan, Ernest B., 600 Medical Arts Bldg. (*Clinical pathology*)
Stone, Murray C., 200 E. Pershing (*Clinical pathology*)

MONTANA

BILLINGS

Martin, Mary Elizabeth, St. Vincent Hospital (*Pathologic anatomy*)

BUTTE

Peterson, Raymond F., Murray Hospital Clinic (*Clinical pathology and pathologic anatomy*)

GREAT FALLS

Hildebrand, Eugene, Box 911 (*Clinical pathology and pathologic anatomy*)
Walker, Thomas F., 503 First Ave., E., (*Clinical pathology and pathologic anatomy*)

MISSOULA

Buchanan, Edwin Graham, St. Patrick's Hospital (*Clinical pathology*)

NEBRASKA

LINCOLN

Tanner, Frank H., Lincoln General Hospital (*Clinical pathology and pathologic anatomy*)

OMAHA

Allen, Pliny, Immanuel Deaconess Hospital (*Clinical pathology and pathologic anatomy*)
Eggers, Harold E., American Cancer Society, 501 Omaha National Bank Bldg. (*Clinical pathology and pathologic anatomy*)
Foster, Miles, 822 S. 68th St. (*Clinical pathology and pathologic anatomy*)
Manning, Ernest T., 1407 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Rubnitz, Abraham, 732 Medical Arts Bldg. (*Clinical pathology*)
Russum, Benjamin C., 306 N. 14th St. (*Clinical pathology and pathologic anatomy*)
Schenken, John R., Nebraska Methodist Hospital (*Clinical pathology and pathologic anatomy*)
Tollman, James P., 42nd St. and Dewey Ave. (*Clinical pathology and pathologic anatomy*)

NEVADA

RENO

Parsons, Lawrence, 235 W. Sixth St. (*Clinical pathology and pathologic anatomy*)
Rea, Stanley L., 235 W. Sixth St. (*Pathologic anatomy*)

NEW HAMPSHIRE

CONCORD

Park, James H., Concord Hospital (*Pathologic anatomy*)

HANOVER

Holyoke, John B., Dartmough Medical School (*Clinical pathology and pathologic anatomy*)
House, Reginald K., Dartmouth Medical School (*Pathologic anatomy*)
Kiarsis, Victor, Dartmouth Medical School (*Pathologic anatomy*)
Miller, Ralph E., 9 Downey Road (*Clinical pathology and pathologic anatomy*)

LACONIA

James, Wendell E , Box 204 (*Clinical pathology and pathologic anatomy*)

MANCHESTER

Biron, Robert E., 337 Notre Dame Ave (*Pathologic anatomy*)
Brooks, Samuel M , 955 Auburn St. (*Clinical pathology and pathologic anatomy*)

ROCHESTER

Norton, Louisa M., Frisbie Memorial Hospital (*Clinical pathology*)

NEW JERSEY

ASBURY PARK

DePons, Isabel S., 501 Grand Ave. (*Pathologic anatomy*)
Pons, Carlos A., 501 Grand Ave. (*Clinical pathology and pathologic anatomy*)

ATLANTIC CITY

Konzelmann, Frank W., Atlantic City Hospital (*Clinical pathology and pathologic anatomy*)

CAMDEN

Platt, William R., West Jersey Hospital (*Clinical pathology and pathologic anatomy*)
Read, W. T., Jr., Cooper Hospital (*Clinical pathology and pathologic anatomy*)

COLLINGSWOOD

Falkinburg, L. W., 501 Park Ave. (*Clinical pathology and pathologic anatomy*)

EAST ORANGE

Albano, Edwin H., 144 So. Harrison St. (*Clinical pathology and pathologic anatomy*)
Fendrick, Edward, 17 Watson Ave. (*Clinical pathology and pathologic anatomy*)
Hulett, Albert G., 20 Hawthorne Ave. (*Clinical pathology and pathologic anatomy*)

GREYSTONE PARK

Sherman, May, New Jersey State Hospital (*Pathologic anatomy*)

HIGHLAND PARK

Moolten, Sylvan E., 103 N. 4th Ave. (*Clinical pathology and pathologic anatomy*)

JERSEY CITY

Alter, Nicholas M., Margaret Hague Hospital (*Clinical pathology and pathologic anatomy*)
Gitlitz, Abraham J., Christ Hospital (*Clinical pathology and pathologic anatomy*)
Price, H. Preston, Medical Center (*Clinical pathology and pathologic anatomy*)

MONTCLAIRE

Work, John L., Mountainside Hospital (*Clinical pathology and pathologic anatomy*)

MOORISTOWN

Bauer, John T., 31 E. Second St. (*Clinical pathology and pathologic anatomy*)

MOUNT PLEASANT

Cassilli, Arturo R., 618 Newark Ave. (*Clinical pathology and pathologic anatomy*)

NEWARK

Antopol, William, 201 Lyons Ave. (*Clinical pathology and pathologic anatomy*)
Byrnes, Elizabeth W., 142 Clinton Ave. (*Clinical pathology*)
Goldberg, Samuel A., Presbyterian Hospital (*Clinical pathology and pathologic anatomy*)
Martland, Harrison S., City Hospital (*Clinical pathology and pathologic anatomy*)
Mermod, Camille, 15 Washington St. (*Clinical pathology and pathologic anatomy*)
Shulman, Murray W., 913 S. 20th St. (*Clinical pathology and pathologic anatomy*)
Yaguda, Asher, 61 Lincoln Park (*Clinical pathology and pathologic anatomy*)

NEW BRUNSWICK

Whitcher, B. R., 44 Broad St. (*Clinical pathology*)

NORTHFIELD

Schmidt, J. Ross, 32 Northfield Plaza (*Clinical pathology and pathologic anatomy*)

ORANGE

Abel, Arthur R., 188 S. Essex Ave. (*Clinical pathology*)
Rose, Salvatore J., 242 Ivy Ct. (*Clinical pathology and pathologic anatomy*)

PASSAIC

Ogden, Michael A., 20 Grove St. (*Clinical pathology and pathologic anatomy*)

PATERSON

Churg, Jacob, Barnert Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Davis, A. H., Paterson General Hospital (*Clinical pathology*)
Kim, Gay Bong, St. Joseph's Hospital (*Clinical pathology and pathologic anatomy*)
Offenkrantz, Frederick M., St. Joseph Hospital (*Clinical pathology*)

PLAINFIELD

Hansen, Harry, Muhlenberg Hospital (*Pathologic anatomy*)

SHORT HILLS

Bernhard, William G., 79 Minnisink Road (*Clinical pathology and pathologic anatomy*)

SOMERVILLE

Gewanter, Aaron, Somerset Hospital (*Clinical pathology and pathologic anatomy*)

TEANECK

Markley, Luther A., Holy Name Hospital (*Clinical pathology*)

TRENTON

Boughton, T. Harris, 928 Carteret Ave. (*Clinical pathology and pathologic anatomy*)
Fluck, David A., 628 W. State St. (*Clinical pathology and pathologic anatomy*)
Rathmell, Thomas K., Mercer Hospital (*Clinical pathology and pathologic anatomy*)

WEEHAWKEN

Braunstein, William P., 1 Bellevue St. (*Clinical pathology and pathologic anatomy*)

NEW MEXICO

ALBUQUERQUE

Beam, Mark P., Van Atta Laboratories, P. O. Box 72 (*Clinical pathology and pathologic anatomy*)

SANTA FE

Margulis, Aaron E., Santa Fe Clinic, Inc., Coronado Bldg. (*Pathologic anatomy*)

NEW YORK

ALBANY

Boland, Joseph B., 308 New Scotland Ave. (*Pathologic anatomy*)
Clemmer, John J., 136 S. Lake Ave. (*Clinical pathology and pathologic anatomy*)
Dalldorf, Gilbert, State of New York Dept. of Health (*Pathologic anatomy*)
Hayes, John J., % L. Johnson, 3 Elk St. (*Pathologic anatomy*)
Marra, John J., 399 State St. (*Clinical pathology and pathologic anatomy*)
Roberts, James B., Albany Medical College (*Clinical pathology and pathologic anatomy*)
Schleifstein, Joseph I., Department of Health, New Scotland Ave. (*Clinical pathology and pathologic anatomy*)
Tompkins, Victor N., New York State Dept. of Health (*Clinical pathology and pathologic anatomy*)
Wright, Arthur W., Albany Medical College (*Clinical pathology and pathologic anatomy*)

AMSTERDAM

Herr, Bayard S., Montgomery County Lab. (*Clinical pathology and pathologic anatomy*)

ASTORIA

Garrow, Irene, 25-15 30th Rd. (*Clinical pathology and pathologic anatomy*)
Werne, Jacob, 25-15 30th Rd. (*Clinical pathology and pathologic anatomy*)

AUBURN

Harris, Arthur E., Cayuga County Laboratory, Metcalfe Bldg. (*Clinical pathology*)

BATAVIA

Preuss, Fred Simon, Veterans Administration Hospital (*Pathologic anatomy*)
Preuss, Fred Simon, Veterans Hospital (*Pathologic anatomy*)
Tannenberg, Joseph, 43 Ellicott Ave. (*Clinical pathology and pathologic anatomy*)

BINGHAMTON

Bergstrom, Victor W., 21 Park Ave. (*Clinical pathology and pathologic anatomy*)
Nelson, Walter C., 7 Crary Ave. (*Clinical pathology and pathologic anatomy*)

BROOKLYN

Baker, Margaret A., Bay Ridge Hospital (*Clinical pathology and pathologic anatomy*)
Bolker, Herman, 264 Westminster Road (*Clinical pathology and pathologic anatomy*)
Bornstein, Siegbert, 1284 President St. (*Clinical pathology and pathologic anatomy*)
Burn, Caspar G., 726 Ocean Ave. (*Clinical pathology and pathologic anatomy*)
Cook, Ward H., Long Island College Hospital (*Clinical pathology and pathologic anatomy*)
Fink, Harold, 700 Ocean Ave. (*Clinical pathology and pathologic anatomy*)
Fox, Lester M., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Friedland, Lester M., 445 Ninth St. (*Pathologic anatomy*)
Gordon, Benjamin S., 1488 President St. (*Pathologic anatomy*)
Grayzel, David M., 105 Winthrop St. (*Pathologic anatomy*)
Grethmann, Wolfgang, 338 34th St. (*Pathologic anatomy*)
Gruenwald, Peter, 335 Henry St. (*Pathologic anatomy*)
Howe, John S., 625 3rd St. (*Clinical pathology and pathologic anatomy*)
Hyman, Malcolm A., 55 Lenox Rd. (*Pathologic anatomy*)
Jacobson, Sheldon A., 460 Lefferts Ave. (*Pathologic anatomy*)
Kantrowitz, Abraham R., 4802 10th Ave. (*Clinical pathology and pathologic anatomy*)
Lederer, Max, 564 E. 23rd St., (*Clinical pathology and pathologic anatomy*)
Mackles, Abraham, 210 Chester St. (*Pathologic anatomy*)
Marino, Edmund R., 504 12th St. (*Pathologic anatomy*)
Marten, M. Edward, 152 Lenox Rd. (*Clinical pathology and pathologic anatomy*)
Meyer, Leo M., 550 E. 16th (*Pathologic anatomy*)
Mitchell, Nathan, Beth-El Hospital (*Clinical pathology and pathologic anatomy*)
Moitrier, William, 1219 Dean St. (*Clinical pathology and pathologic anatomy*)
Pennell, S., 4711 12th Ave. (*Clinical pathology*)
Polayes, Silik, 414 Sterling Place (*Clinical pathology and pathologic anatomy*)
Rakieten, Morris L., 335 Henry St. (*Clinical pathology*)
Rosenblatt, Philip, 142 Joralemon (*Pathologic anatomy*)
Rosenthal, Lazar, 353 Ocean Ave. (*Clinical pathology*)
Sherman, Irving, 1466 49th St. (*Clinical pathology*)
Wachstein, Maximilian, 782 E. 17th St. (*Clinical pathology and pathologic anatomy*)
Wiener, Alexander S., 64 Rutland Road (*Clinical pathology*)

BUFFALO

Becker, Charles F., 516 Lafayette St. (*Pathologic anatomy*)
Jacobs, William F., 310 W. Utica (*Pathologic anatomy*)
Postoloff, Anthony V., 24 Seminole Pkwy. (*Pathologic anatomy*)
Sanes, Samuel, Ed. T. Meyer Hospital (*Pathologic anatomy*)
Tannhauser, Siegfried, 563 Riley St. (*Clinical pathology and pathologic anatomy*)
Warwick, Margaret, 610 Potomac Ave. (*Clinical pathology and pathologic anatomy*)

CENTRAL ISLIP

Trygstad, Reidar, Central Islip (*Clinical pathology and pathologic anatomy*)

CLIFTON SPRINGS

Copeland, Glenn J., Clifton Springs San. & Clinic (*Clinical pathology*)

COOPERSTOWN

Hawn, Clinton Van Zandt, Otsego County Laboratory (*Pathology anatomy*)

CORONA

Penke, Madeline, 104-27 41st St. (*Clinical pathology and pathologic anatomy*)

CORTLAND

Wall, William A., 105 N. Main St. (*Clinical pathology*)

EAST WILLISTON

deVeer, J. Arnold, 33 Meritoria Drive (*Clinical pathology and pathologic anatomy*)

ELMHURST

Barland, Samuel, 40-66 Ithaca St. (*Clinical pathology and pathologic anatomy*)

ELMIRA

Cohen, Samuel E., Arnot-Ogden Memorial Hospital (*Pathologic anatomy*)

FAR ROCKAWAY

Handelman-Kalashnikoff, Pauline, 918 Cornaga Ave. (*Clinical pathology*)

FLUSHING

Gettinger, Raymond, 73-40 195th St. (*Clinical pathology and pathologic anatomy*)

FOREST HILLS

Feigin, Irvin, 7272 112th St. (*Clinical pathology and pathologic anatomy*)
Meyer, Elizabeth, 112-41 72nd Road (*Pathologic anatomy*)

GLENS FALLS

Maslon, Morris, 88 Park St. (*Clinical pathology and pathologic anatomy*)

GREAT NECK

Marshall, Louis E., 10 Welwyn Road, Apt. 1 F (*Clinical pathology and pathologic anatomy*)

HEMPSTEAD, LONG ISLAND

Curphey, Theodore J., Meadowbrook Hospital (*Clinical pathology and pathologic anatomy*)

HUNT

Popoff, Nicholas W., R. F. D. #1 (*Clinical pathology and pathologic anatomy*)

HUNTINGTON, LONG ISLAND

Holleb, Herbert B., Huntington Hospital (*Clinical pathology and pathologic anatomy*)

ITHACA

Ferris, Henry W., Tompkins County Memorial Hospital (*Clinical pathology and pathologic anatomy*)

JACKSON HEIGHTS

Santora, Edward, 32-56 83rd St. (*Clinical pathology and pathologic anatomy*)

JAMAICA, LONG ISLAND

Angrist, Alfred, 166-19 89th Ave. (*Clinical pathology and pathologic anatomy*)

JAMESTOWN

Loeffler, Margaret, Municipal Laboratory (*Pathologic anatomy*)
Weedon, Frederick R., Municipal Laboratory (*Clinical pathology and pathologic anatomy*)

JOHNSON CITY

Stevens, Dorothy L., Charles S. Wilson Memorial Hospital (*Clinical pathology and pathologic anatomy*)

JORDAN

Van Horn, Herman H., (*Clinical pathology*)

KEW GARDENS

Rosen, Samuel, 118-09 83rd Ave. (*Pathologic anatomy*)

KINGS PARK

Priestman, Gordon, Kings Park State Hospital (*Clinical pathology and pathologic anatomy*)

KINGSTON

Taylor, J. S., 400 Broadway (*Clinical pathology and pathologic anatomy*)

LAURELTON, LONG ISLAND

Barnard, Robert D., 138-48 231st St. (*Clinical pathology*)

LONG ISLAND CITY

Chapman, Irving, 41-15 45th Street (*Clinical pathology and pathologic anatomy*)
Siegel, Henry, 18-45 21st Road (*Clinical pathology and pathologic anatomy*)

MIDDLETOWN

Kelley, William E., State Hospital (*Clinical pathology and pathologic anatomy*)

MINEOLA

Inda, Frank A., Nassau Hospital (*Clinical pathology and pathologic anatomy*)

MONROE

Hala, William W. (*Clinical pathology and pathologic anatomy*)

MOUNT MORRIS

Mathias, E. E. M., Mt. Morris Tuberculosis Hospital (*Pathologic anatomy*)

MOUNT VERNON

Gross, Stanley, 24 Claremont Ave. (*Clinical pathology*)
Sharnoff, J. George, Mt. Vernon Hospital (*Clinical pathology and pathologic anatomy*)

NEWBURGH

Summerill, Frederick, 70 Dubois (*Clinical pathology and pathologic anatomy*)
Wescott, Adeline M., 202 Grand St. (*Clinical pathology and pathologic anatomy*)

NEW ROCHELLE

Denton, James W., 16 Guion Place (*Pathologic anatomy*)
Fruend-Klemperer, Margit, 18 Lester Place (*Clinical pathology and pathologic anatomy*)

NEW YORK

Adler, Jacob, Mount Sinai Hospital (*Pathologic anatomy*)
Allen, Arthur C., 235 E. 22nd St. (*Clinical pathology and pathologic anatomy*)
Andersen, Dorothy H., Babies Hospital (*Clinical pathology and pathologic anatomy*)
Aronoff, Rosa, 36 Washington Square, W. (*Clinical pathology and pathologic anatomy*)
Aronson, William, 1840 Grand Concourse (*Clinical pathology and pathologic anatomy*)
Beckett, Ronald S., Montefiore Hospital, E. Gunhill Rd. (*Clinical pathology and pathologic anatomy*)
Bernheim, Alice I., 65 E. 96th St. (*Pathologic anatomy*)
Bevans, Margaret, 1st Columbia Research Division Goldwater Memorial Hospital (*Pathologic anatomy*)
Block, Nathan, 1082 Park Ave. (*Clinical pathology and pathologic anatomy*)
Brandes, Walter W., Roosevelt Hospital (*Clinical pathology and pathologic anatomy*)
Brody, Henry, Beth Israel Hospital (*Pathologic anatomy*)
Brown, Chester R., 4418 Waldo Ave. (*Pathologic anatomy*)
Brown, Gregory N., New York University College of Dentistry (*Clinical pathology and pathologic anatomy*)
Caes, Henry J., New York Medical College (*Pathologic anatomy*)
Clark, Eugene, 133 E. 58th St. (*Pathologic anatomy*)
Clarke, B. Earle, St. Luke's Hospital (*Clinical pathology and pathologic anatomy*)
Coblenz, Aaron, 500 E. 81st St. (*Clinical pathology and pathologic anatomy*)
Cocheu, Lindsley F., 20 E. 106th St. (*Clinical pathology*)
Cohen, Hilliard, Mount Sinai Hospital (*Clinical pathology and pathologic anatomy*)
Connery, Joseph E., 138 E. 36th St. (*Clinical pathology*)
Darlington, Charles G., 477 First Ave. (*Clinical pathology and pathologic anatomy*)
DeAngeles, C. E., St. John's Riverside Hospital (*Clinical pathology and pathologic anatomy*)
Dolgopol, Vern B., 610 W. 115th St. (*Clinical pathology and pathologic anatomy*)
Eggston, Andrew A., 653 Park Ave. (*Clinical pathology and pathologic anatomy*)
Ehrenreich, Theodore, 400 E. 57th St. (*Pathologic anatomy*)
Eisen, J. Milton, 630 W. 168th St. (*Pathologic anatomy*)
Eros, Gedeon, 722 W. 168th St. (*Pathologic anatomy*)
Farinacci, Charles J., 1st Army Lab., 90 Church St. (*Clinical pathology and pathologic anatomy*)
Fein, Maxwell J., 30 E. 40th St. (*Clinical pathology and pathologic anatomy*)
Felsen, Joseph, 120 E. 39th St. (*Clinical pathology and pathologic anatomy*)
Fitzgerald, Patrick J., York Ave. and 68th St. (*Clinical pathology and pathologic anatomy*)
Flynn, Joseph E., 630 W. 168th St. (*Pathologic anatomy*)
Foot, N. Chandler, 1300 York Ave. (*Pathologic anatomy*)
Foote, Frank W., Jr., 444 E. 68th St. (*Pathologic anatomy*)
Foucar, F. H., Col., M. C., U. S. A. First Army Lab. (*Clinical pathology and pathologic anatomy*)
Frantz, Virginia K., 630 W. 168th St. (*Pathologic anatomy*)
Garber, C. Zant, 420 59th St., E. (*Clinical pathology and pathologic anatomy*)
Geiger, Jacob, 1070 Park Ave. (*Clinical pathology*)
Gerber, Isadore E., 1175 Park Ave. (*Clinical pathology and pathologic anatomy*)
Ginzler, Arthur M., 565 Manhattan Ave. (*Pathologic anatomy*)
Gladstone, Sidney A., 345 W. 50th St. (*Clinical pathology and pathologic anatomy*)
Gonzales, Thomas A., 730 Ft. Washington Ave. (*Pathologic anatomy*)
Graef, Irving, 477 First Ave. (*Pathologic anatomy*)
Grishman, Edith, 25 E. 98th St. (*Pathologic anatomy*)
Gruenwald, Lotte, 1 E. 100th Street (*Pathologic anatomy*)
Halpern, Milton, 106 E. 85th St. (*Pathologic anatomy*)
Higgins, George K., New York Medical College, 1 E. 105th St. (*Pathologic anatomy*)
Hillman, Oliver S., 140 E. 54th St. (*Clinical pathology and pathologic anatomy*)
Hochman, Charles H., 1749 Grand Concourse (*Clinical pathology and pathologic anatomy*)
Jaffe, Henry L., Hospital for Joint Diseases (*Clinical pathology and pathologic anatomy*)
Jessup, David S. D., 411 W. 114th St. (*Clinical pathology and pathologic anatomy*)
Kean, Benjamin H., 710 Park Ave. (*Clinical pathology and pathologic anatomy*)
Kellner, Aaron, 1300 York Ave. (*Pathologic anatomy*)
Keston, Homer D., 630 W. 168th St. (*Pathologic anatomy*)
Klemperer, Paul, Mt. Sinai Hospital (*Clinical pathology and pathologic anatomy*)
Knox, Leila C., St. Luke's Hospital (*Clinical pathology and pathologic anatomy*)
Kogen, Vitali, 235 Ft. Washington Ave. (*Pathologic anatomy*)
Kopel, Moses, 1063 Morris Ave. (*Clinical pathology and pathologic anatomy*)
Kritzler, Robert A., 622 West 168th St. (*Pathologic anatomy*)
Lattes, Raffaele, 630 W. 168th St. (*Pathologic anatomy*)
Lefkowitz, Louis L., 1235 Grand Concourse (*Pathologic anatomy*)
Levine, Jacob, 235 E. 196th St. (*Clinical pathology and pathologic anatomy*)

Lichtenstein, Louis, 1919 Madison Ave. (*Pathologic anatomy*)
Lisa, James R., City Hospital, Welfare Island (*Clinical pathology and pathologic anatomy*)
Manheims, Perry J., 830 Park Ave. (*Clinical pathology*)
Mendeloff, Joseph, 2115 Ryer Ave. (*Pathologic anatomy*)
Millman, Louis J., 2488 Grand Concourse (*Pathologic anatomy*)
Moss, Leo D., 1 E. 100th St. (*Clinical pathology and pathologic anatomy*)
Olcott, Charles T., 1300 York Ave. (*Pathologic anatomy*)
Olivetti, Renzo G , 301 E. 21st St (*Pathologic anatomy*)
Paltauf, Rudolf, 344 W. 72nd St. (*Pathologic anatomy*)
Pearce, John M., 525 E. 68th St. (*Pathologic anatomy*)
Pincus, Julius, 300 West End Ave. (*Clinical pathology and pathologic anatomy*)
Plaut, Alfred, Beth Israel Hospital (*Clinical pathology and pathologic anatomy*)
Ravid, Jacob M., 1125 Park Ave. (*Clinical pathology and pathologic anatomy*)
Richter, Maurice N., 303 E. 20th St. (*Clinical pathology and pathologic anatomy*)
Rohdenburg, George L., 530 Park Ave. (*Clinical pathology and pathologic anatomy*)
Rosenthal, Julius, 114 E. 84th St. (*Clinical pathology and pathologic anatomy*)
Rothstein, Isadore, Bronx Hospital (*Clinical pathology and pathologic anatomy*)
Rottino, Antonio, 2735 Henry Hudson Highway (*Pathologic anatomy*)
Saccone, Andrea, 334 E. 116th St. (*Clinical pathology and pathologic anatomy*)
St. George, Armin V., 400 E. 20th St. (*Clinical pathology and pathologic anatomy*)
Sala, Angelo M., N. Y. C. Cancer Institute, Welfare Island (*Pathologic anatomy*)
Seecof, David P., 1970 Daly Ave. (*Pathologic anatomy*)
Selzer, Isidore, New York City Hospital (*Pathologic anatomy*)
Silverman, Gertrude, 301 E. 21st St. (*Clinical pathology and pathologic anatomy*)
Silverman, I. Jerome, 910 Grand Concourse (*Clinical pathology and pathologic anatomy*)
Smith, H. P., 630 W. 168th St. (*Clinical pathology and pathologic anatomy*)
Smith, Lawrence W., 119 E. 26th St. (*Clinical pathology and pathologic anatomy*)
Solomon, Cyril, French Hospital, 330 W. 30th St. (*Pathologic anatomy*)
Spain, David M., Bellevue Hospital (*Pathologic anatomy*)
Speer, Francis D., 20 E. 106th St. (*Clinical pathology and pathologic anatomy*)
Spitz, Henry, Bellevue Hospital, (*Pathologic anatomy*)
Spitz, Sophie, 235 E. 22nd Street (*Pathologic anatomy and clinical pathology*)
Stanford, Addie D., Ontario Wayne Co. (*Clinical pathology*)
Stewart, Fred W., 444 E. 68th St. (*Clinical pathology and pathologic anatomy*)
Stillman, Ralph G., 525 E. 68th St. (*Clinical pathology*)
Stohr, Grete, 10 W. 74th St. (*Pathologic anatomy*)
Stout, Arthur P., 630 W. 168th St. (*Pathologic anatomy*)
Stump, David J., Postgraduate Hospital (*Clinical pathology and pathologic anatomy*)
Taub, Jacob, 1488 Metropolitan Ave. (*Clinical pathology and pathologic anatomy*)
Vance, Benjamin M., 400 E. 29th St. (*Pathologic anatomy*)
Vogel, Peter, 51 E. 90th St. (*Clinical pathology*)
Von Glahn, William C., 477 First Ave. (*Pathologic anatomy*)
Weintraub, Solomon, 350 Central Park W. (*Pathologic anatomy*)
Wilens, Sigmund, University and Bellevue Hospital Medical College (*Pathologic anatomy*)
Wolf, Abner, 630 W. 168th St. (*Pathologic anatomy*)
Wood, Francis Carter, St. Luke's Hospital (*Clinical pathology and pathologic anatomy*)
Wozencraft, Jean Paul, 444 E. 68th St. (*Clinical pathology and pathclogic anatomy*)
Youland, William E., 1 East 105th St. (*Clinical pathology and pathologic anatomy*)
Zak, Frederick G., Mount Sinai Hospital (*Pathologic anatomy*)
Zimmerman, Harry M., Montefiore Hospital (*Pathologic anatomy*)

NYACK

Dessau, Frederick I., Route 9, Upper Grandview (*Pathologic anatomy*)

ONEONTA

Loesch, John, Homer Folks Tuberculosis Hospital (*Pathologic anatomy*)

PORTVILLE

Smith, J. Howard, 32 Maple Ave. (*Clinical pathology and pathologic anatomy*)

POUGHKEEPSIE

Ferraro, Louis R., St. Francis Hospital (*Clinical pathology and pathologic anatomy*)
Peckham, A. L., Vassar Brothers Hospital (*Clinical pathology and pathologic anatomy*)

ROCHESTER

Bohrod, Milton G., 501 W. Main St. (*Clinical pathology and pathologic anatomy*)
Brown, Herbert R., 215 S. Goodman St. (*Clinical pathology and pathologic anatomy*)
Bullard, H. Hays, Genesee Hospital (*Clinical pathology and pathologic anatomy*)
Gordon, Louis Z., Highland Hospital of Rochester (*Clinical pathology and pathologic anatomy*)
Hawkins, William Bruce, 260 Crittenden Blvd. (*Pathologic anatomy*)
Lindsay, S. T., St. Mary's Hospital (*Clinical pathology and pathologic anatomy*)
Mider, G. Burroughs, Strong Memorial Hospital (*Pathologic anatomy*)
Scott, James K., Rochester Hospital (*Pathologic anatomy*)
Thomas, Walter S., 1321 Highland Ave. (*Clinical pathology and pathologic anatomy*)
Whipple, George H., 260 Crittenden Blvd. (*Clinical pathology and pathologic anatomy*)

RYE

Loder, Margaret, 926 Boston Post Road (*Clinical pathology and pathologic anatomy*)

SARANAC LAKE

Vorwald, Arthur J., Saranac Laboratory, Box 551 (*Clinical pathology and pathologic anatomy*)

SARATOGA SPRINGS

Lebowich, Joseph, Saratoga County Laboratory, Saratoga Hospital (*Pathologic anatomy*)

SCARSDALE

Meeker, Louise H., 1245 Post Road (*Pathologic anatomy*)
Russell, Hollis K., 1234 Post Road (*Clinical pathology and pathologic anatomy*)

SCHENECTADY

Kellert, Ellis, Ellis Hospital (*Clinical pathology and pathologic anatomy*)

STATEN ISLAND

Auerbach, Oscar, 121 Hamden Ave. (*Pathologic anatomy*)
Sophian, Lawrence, U. S. Marine Hospital (*Clinical pathology and pathologic anatomy*)
Weiss, Leo, Halloran Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

SYRACUSE

Dick, Herman J., Syracuse University College of Medicine (*Clinical pathology and pathologic anatomy*)
Ferguson, J. Howard, 766 Irving Ave. (*Pathologic anatomy*)
Reifenstein, George H., Syracuse University (*Pathologic anatomy*)
Weiskotten, Herman G., 766 Irving Ave. (*Pathologic anatomy*)

TROY

Curtis, Stephen H., 127 Congress (*Clinical pathology*)
Gardner, Louise Trent, 1.2 Georgian Terrace, Rensselaerwyck (*Clinical pathology and pathologic anatomy*)
Kosinski, Alexander A., Leonard Hospital (*Pathologic anatomy*)

UTICA

Walker, Gerald C., St. Elizabeth Hospital (*Pathologic anatomy*)

VALHALLA

McCullough, Kendrick, Grasslands Hospital (*Pathologic anatomy*)

WATERTOWN

Walker, Thomas Tipton, 546 Woolworth Building (*Clinical pathology and pathologic anatomy*)

WINGDALE

Cares, Reuben, Harlem Valley State Hospital (*Clinical pathology and pathologic anatomy*)

WOODHAVEN

Bevilacqua, Michael, 80-31 88th Ave. (*Clinical pathology and pathologic anatomy*)

NORTH CAROLINA

ASHEVILLE

Derrick, W. A., Norburn Hospital and Clinic (*Pathologic anatomy*)
Peasley, E. D., Mission Hospital (*Clinical pathology and pathologic anatomy*)

CHAPEL HILL

Brinkhous, Kenneth M., School of Medicine (*Clinical pathology and pathologic anatomy*)

CHARLOTTE

Byrnes, Thomas H., 403 N. Tryon St. (*Clinical pathology and pathologic anatomy*)
Kimmelstiel, Paul, Charlotte Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Todd, Lester C., 401 N. Tryon St. (*Clinical pathology and pathologic anatomy*)

DURHAM

Black-Schaffer, Bernard, Duke University Hospital (*Pathologic anatomy*)
Cuttino, John T., Box 3128, Duke Hospital (*Pathologic anatomy*)
Dickson, James Gillespie, Box 3440, Duke Hospital (*Clinical pathology*)
Erickson, Cyrus C., Duke University Hospital (*Pathologic anatomy*)
Forbus, Wiley D., Duke University Hospital (*Clinical pathology and pathologic anatomy*)
Gunter, June U., Watts Hospital (*Clinical pathology and pathologic anatomy*)
Margolis, George, Duke University Hospital (*Clinical pathology and pathologic anatomy*)

GREENSBORO

Lennon, Hershel C., 116 Jefferson Bldg. (*Clinical pathology and pathologic anatomy*)

WILMINGTON

Herzberg, Mortimer, 2517 Princess St. (*Clinical pathology and pathologic anatomy*)

WINSTON-SALEM

Carpenter, Coy C., Bowman Gray School of Medicine (*Clinical pathology and pathologic anatomy*)
Morehead, Robert Page, Bowman Gray School of Medicine (*Pathologic anatomy*)

NORTH DAKOTA

BISMARCK

Larson, Leonard W., 221 Fifth St. (*Clinical pathology and pathologic anatomy*)

OHIO

AKRON

Baker, Margaret R., Children's Hospital (*Clinical pathology and pathologic anatomy*)
Catron, Lloyd F., City Hospital (*Pathologic anatomy*)
Dochat, George R., Peoples Hospital (*Clinical pathology and pathologic anatomy*)
Proskauer, George S., St. Thomas Hospital (*Pathologic anatomy*)

CANTON

Henderson, Donald G., Aultman Hospital (*Clinical pathology and pathologic anatomy*)
Rosedale, Raymond S., 610 Market Ave. (*Pathologic anatomy*)
Shaweker, Max, Mercy Hospital (*Clinical pathology*)

CINCINNATI

Brill, Robert, St. Mary's Hospital, 816 Betts St. (*Clinical pathology and pathologic anatomy*)
Dutra, Frank R., University of Cincinnati (*Pathologic anatomy*)
Gall, Edward A., Cincinnati General Hospital (*Pathologic anatomy*)
Gannon, John R., 234 Ehrman Ave. (*Clinical pathology and pathologic anatomy*)
Germain, Wm McKee, Good Samaritan Hospital (*Clinical pathology and pathologic anatomy*)
Wasserman, Philip, Jewish Hospital (*Clinical pathology and pathologic anatomy*)

CLEVELAND

Chamberlin, William B., Jr., St. Alexis Hospital (*Clinical pathology and pathologic anatomy*)
Domingues, Rafael, 11311 Shaker Blvd. (*Clinical pathology and pathologic anatomy*)
Eitzen, Oliver, St. Alexis Hospital, Broadway & McBride (*Pathologic anatomy*)
Goodsitt, Edward, Huron Road Hospital (*Clinical pathology and pathologic anatomy*)
Harmos, Oscar, U. S. Marine Hosp., Fairhill Road and E. 124th St. (*Clinical pathology and pathologic anatomy*)
Karsner, Howard T., 2085 Adelbert Rd. (*Clinical pathology and pathologic anatomy*)
Kinney, Thomas D., 3395 Scranton Rd. (*Pathologic anatomy*)
Kline, Benjamin S., Mt. Sinai Hospital (*Clinical pathology and pathologic anatomy*)
Koletsky, Simon, 2085 Adelbert Rd. (*Clinical pathology and pathologic anatomy*)
Koster, E. Frederick, St. John's Hospital (*Pathologic anatomy*)
Lund, Herbert, University Hospital (*Clinical pathology and pathologic anatomy*)
Mackrell, John S., Jr., Fairview Park Hospital (*Pathologic anatomy*)
Orbison, J. L., 2085 Adelbert Rd. (*Pathologic anatomy*)
Rehbock, Donald J., 2315 E. 22nd (*Clinical pathology and pathologic anatomy*)
Segal, Abraham J., St. Luke's Hospital (*Pathologic anatomy*)
Sneiderman, Harry, 968 Hanna Bldg. (*Clinical pathology*)
Sunderman, F. William, Cleveland Clinic, Euclid at 93rd (*Clinical pathology*)
Taylor, Richard C., 3395 Scranton Road (*Pathologic anatomy*)
Young, Anna May, 1800 E. 105th St. (*Clinical pathology and pathologic anatomy*)

CLEVELAND HEIGHTS

Bayless, Francis, 2316 Delaware St. (*Clinical pathology and pathologic anatomy*)
Hazard, J. B., Cleveland Clinic (*Clinical pathology and pathologic anatomy*)
Leuchtenberger, Rudolf, 12345 Cedar Rd. (*Pathologic anatomy*)

COLUMBUS

Davidson, Horace B., 267 E. Broad St. (*Clinical pathology and pathologic anatomy*)
Fidler, Roswell S., 700 N. Park St. (*Clinical pathology and pathologic anatomy*)
Milliser, Russell V., Ohio State University (*Pathologic anatomy*)
Reinhart, Harry L., Starling-Loving U. Hospital (*Clinical pathology and pathologic anatomy*)
Schlumberger, Hans G., Ohio State University (*Clinical pathology and pathologic anatomy*)
Von Hamm, Emmerich, Ohio State University (*Clinical pathology and pathologic anatomy*)

CUYAHOGA FALLS

Potter, Frederick C., 826 Chestnut Blvd. (*Clinical pathology and pathologic anatomy*)

DAYTON

Oosting, Melvin, Miami Valley Hospital (*Clinical pathology and pathologic anatomy*)
Payne, F. C., 880 Fidelity Medical Bldg. (*Clinical pathology and pathologic anatomy*)
Simpson, Walter M., 103 Spirea Dr. (*Clinical pathology and pathologic anatomy*)

ELYRIA

Chomet, Bernard, Elyria Hospital (*Clinical pathology and pathologic anatomy*)

HAMILTON

Lande, Kurt E., 837 Main St. (*Pathologic anatomy*)

LIMA

Hemsath, F. A., P.O. Box 1031 (*Clinical pathology and pathologic anatomy*)

LORAIN

Chesner, Charles, St. Joseph's Hospital (*Clinical pathology and pathologic anatomy*)

MANSFIELD

Hathaway, Burr M , General Hospital (*Clinical pathology and pathologic anatomy*)

SALEM

Falkenstein, Alfons F., 641 Perry St. (*Clinical pathology and pathologic anatomy*)

SPRINGFIELD

Hunter, Mary P., Springfield City Hospital (*Clinical pathology and pathologic anatomy*)

TOLEDO

Burns, Edward L., Mercy Hospital (*Pathologic anatomy*)
Ramsey, Thomas L., 225 Michigan (*Clinical pathology and pathologic anatomy*)
Rhoden, Alfred E., Flower Hospital, Cherry St. (*Clinical pathology and pathologic anatomy*)
Rich, George T., Maumee Valley Hospital (*Pathologic anatomy*)
Steinberg, Bernhard, Toledo Hospital (*Clinical pathology and pathologic anatomy*)
Vidoli, Marino F., St. Vincent's Hospital (*Pathologic anatomy*)
Zbinden, Theodore, 706 Madison Ave (*Clinical pathology*)

WARREN

Scullard, Garner, Warren City Hospital (*Clinical pathology and pathologic anatomy*)

YOUNGSTOWN

Collier, Wm. Dean, 1044 Belmont Ave. (*Clinical pathology and pathologic anatomy*)
Giffen, Horace K., Youngstown Hospital Assn. (*Pathologic anatomy*)
Rappoport, Arthur E., Youngstown Hospital (*Clinical pathology and pathologic anatomy*)
Raven, Clara, 2219 Goleta Avenue (*Clinical pathology*)

OKLAHOMA

BARTLESVILLE

Chamberlin, Elizabeth, Memorial Hospital (*Clinical pathology*)

OKLAHOMA CITY

Halpert, Bela, University Hospital (*Clinical pathology and pathologic anatomy*)
Hopps, Howard C., 801 E. 13th St. (*Pathologic anatomy*)
Keller, Wilbur Floyd, Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)

SHAWNEE

Owen, James N., Jr., Anderson, Carson & Hughes Clinic (*Pathologic anatomy*)

TULSA

Palik, Emil E., St John's Hospital (*Clinical pathology and pathologic anatomy*)

OREGON

EUGENE

Furrer, Emil D., 132 E. Broadway (*Clinical pathology*)

PORTLAND

Crynes, Sylvester, 705 Standard Bldg. (*Clinical pathology*)
Foskett, Herbert H , 1020 S. W. Taylor St. (*Clinical pathology and pathologic anatomy*)
Frisch, Arthur W., University of Oregon Medical School (*Pathologic anatomy*)
Hunter, Warren C., University of Oregon Medical School (*Clinical pathology and pathologic anatomy*)
Johnston, Robert D., University of Oregon Medical School (*Pathologic anatomy*)
Lehman, William, Good Samaritan Hospital (*Clinical pathology and pathologic anatomy*)
Losli, Ernest J., 4505 N. E. Royal Court (*Clinical pathology and pathologic anatomy*)
Manlove, Charles H., Good Samaritan Hospital (*Clinical pathology and pathologic anatomy*)
Menne, Frank R., St. Vincent's Hospital (*Clinical pathology and pathologic anatomy*)
Minckler, Jeff, Providence Hospital (*Pathologic anatomy*)
Queen, Frank B., 3181 S. W. Marquan Hill Rd. (*Pathologic anatomy*)
Richardson, Howard L., Crime Detection Laboratory, Oregon University (*Pathologic anatomy*)
Sneeden, Vinton D , University of Oregon Medical School (*Pathologic anatomy*)

SALEM

Lidbeck, Wm. L., 390 State St. (*Clinical pathology and pathologic anatomy*)

PENNSYLVANIA

ABINGTON

Eiman, John, Abington Memorial Hospital (*Clinical pathology and pathologic anatomy*)

ALLENTOWN

Reitz, Charles B., Allentown State Hospital (*Clinical pathology*)
Stader, Donald E., Sacred Heart Hospital (*Pathologic anatomy*)
Wenner, John J., 44 N. 13th Street (*Clinical pathology and pathologic anatomy*)

ARDMORE

Belk, William P., Times-Medical Bldg. (*Clinical pathology and pathologic anatomy*)

ASPINWALL

Ackerman, Milton, Veterans Administration Hospital (*Pathologic anatomy*)
Bennett, Webster W., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Brilmyer, George J., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

BETHLEHEM

Gasper, Istvan A., 1104 W. Market St. (*Clinical pathology and pathologic anatomy*)

BRADDOCK

Lamb, Harold H., 741 Braddock Ave. (*Clinical pathology*)

BRYN MAWR

McGraw, John J., Jr., Bryn Mawr Hospital (*Pathologic anatomy*)
Sappington, S. W., Box 528 (*Clinical pathology and pathologic anatomy*)
Strumia, Max M., Bryn Mawr Hospital (*Clinical pathology and pathologic anatomy*)

CHESTER

Hollingsworth, I. Pemberton P., 411 N. Walnut St., West. (*Clinical pathology and pathologic anatomy*)

COATESVILLE

Lochhead, Harrie Burton, Veterans Administration (*Clinical pathology*)

CYNWYD

Haentze, Frederick E., 307 Bryn Mawr Ave. (*Clinical pathology*)

DANVILLE

Hunt, Henry F., Geisinger Memorial Hospital (*Clinical pathology and pathologic anatomy*)

DARBY

Valdes-Dapena, Antonio M., Fitzgerald-Mercy Hospital (*Pathologic anatomy*)

EASTON

Larkum, Newton W , Easton Hospital (*Clinical pathology*)
Zillensen, Fred O., Drake Building (*Clinical pathology and pathologic anatomy*)

EAST STROUDSBURG

Lanyon, W. L , Rosencranz Hospital (*Clinical pathology and pathologic anatomy*)

ERDENHEIM

Schradieck, Constant E., 116 Haws Lane (*Clinical pathology and pathologic anatomy*)

GETTYSBURG

Stewart, Henry A., 230 Baltimore St. (*Clinical pathology*)

HARRISBURG

Boughton, Thelma G., Harrisburg State Hospital (*Pathologic anatomy*)
Forrester, James S., Polyclinic Hospital (*Clinical pathology and pathologic anatomy*)

HAZLETON

Foldes, Julius, Hazleton State Hospital (*Clinical pathology and pathologic anatomy*)

HOMESTEAD

Barbrow, A. L., Homestead Hospital (*Clinical pathology and pathologic anatomy*)

IRWIN

McClellan, Robert H., Pennsylvania and Locust St. (*Clinical pathology and pathologic anatomy*)

KINGSTON

Baker, Theodore, Jr., Nesbitt Memorial Hospital (*Clinical pathology and pathologic anatomy*)

LANCASTER

Heid, George J., Jr., Lancaster General Hospital (*Pathologic anatomy*)

LANSDOWNE

de Rivas, Damaso, 106 E. Plumstead Ave. (*Clinical pathology and pathologic anatomy*)

LEWISTOWN

Schaefer, Leroy W., Lewistown Hospital (*Clinical pathology and pathologic anatomy*)

McKEESPORT

Menlowe, Patterson M., McKeesport Hospital (*Clinical pathology and pathologic anatomy*)

MEADVILLE

Burt, Robert C., Meadville City Hospital (*Clinical pathology and pathologic anatomy*)

NARBERTH

Smith, Edward B., 207 Lantwyn Lane (*Clinical pathology and pathologic anatomy*)

NEWCASTLE

Goodpastor, William E., Jameson Memorial Hospital (*Pathologic anatomy*)

NORRISTOWN

Simpson, John C., 920 Swede St. (*Clinical pathology and pathologic anatomy*)

PHILADELPHIA

Adamkiewicz, L. L., Graduate Hospital, 19th & Lombard Sts. (*Clinical pathology and pathologic anatomy*)
Aegerter, Ernest E., Temple University Medical School (*Clinical pathology and pathologic anatomy*)
Aronson, Joseph D., Henry Phipps Institute, Seventh & Lombard Sts. (*Clinical pathology and pathologic anatomy*)
Austin, J. Harold, 36th & Spruce St. (*Clinical pathology*)
Brown, Clark E., Lankenau Hospital (*Clinical pathology and pathologic anatomy*)
Brown, Claude P., 1930 Chestnut St (*Clinical pathology and pathologic anatomy*)
Bucher, Carl J., 271 S. 15th St. (*Clinical pathology and pathologic anatomy*)
Case, Eugene A., Graduate Hospital (*Clinical pathology and pathologic anatomy*)
Ciconne, Emmet F., 8050 Holme Ave. (*Clinical pathology and pathologic anatomy*)
Clark, Jefferson H., Philadelphia General Hospital (*Clinical pathology and pathologic anatomy*)
Cook, Hunter S., Hahnemann Hospital (*Clinical pathology and pathologic anatomy*)
Cope, Thomas A., Jr., 81 W. Upsal St. (*Clinical pathology and pathologic anatomy*)
Crane, A. R , Pennsylvania Hospital (*Clinical pathology and pathologic anatomy*)
Crawford, Baxter, 10th and Sansom St., Jefferson Hospital (*Clinical pathology and pathologic anatomy*)
Custer, R. Philip, Director of Laboratories, Presbyterian Hospital (*Clinical pathology and pathologic anatomy*)
Donnelly, Andrew J., American Oncologic Hospital (*Clinical pathology and pathologic anatomy*)

Ehrich, William E., Pennsylvania University School of Medicine (*Pathologic anatomy*)
Fite, Franklin, 8th & Spruce Sts. (*Clinical pathology and pathologic anatomy*)
Fox, Herbert, University of Pennsylvania (*Clinical pathology and pathologic anatomy*)
Froio, Gregory F., 235 N. 15th Street (*Clinical pathology and pathologic anatomy*)
Gault, Edwin S , Temple University School of Medicine (*Clinical pathology and pathologic anatomy*)
Geiss, Mollie A., Woman's Medical College (*Pathologic anatomy*)
Ginsburg, Gershon, 1936 N. Seventh St. (*Clinical pathology*)
Gregory, John E., 235 N. 15th Street (*Clinical pathology and pathologic anatomy*)
Hastings, Willard S., Jeanes Hospital (*Clinical pathology and pathologic anatomy*)
Herbut, Peter A., Jefferson Hospital (*Clinical pathology and pathologic anatomy*)
Horn, Robert C., University of Pennsylvania (*Pathologic anatomy*)
Horneff, J. Arthur, 230 N. Broad St. (*Clinical pathology*)
Ingleby, Helen, Jewish Hospital (*Pathologic anatomy*)
Jenny, Florence S., Women's Medical College (*Pathologic anatomy*)
Krumbhaar, Edward B., University of Pennsylvania Medical School (*Clinical pathology and pathologic anatomy*)
Lieber, Marshall M., Jefferson Medical College (*Pathologic anatomy*)
Long, Esmond R., Henry Phipps Institute (*Clinical pathology and pathologic anatomy*)
Lucké, Balduin, University of Pennsylvania Medical School (*Pathologic anatomy*)
Lynch, Frank B., Germantown Dispensary and Hospital (*Clinical pathology and pathologic anatomy*)
McCutcheon, Morton, University of Pennsylvania Medical School (*Pathologic anatomy*)
Martens, Vernon, University of Pennsylvania Medical School (*Clinical pathology and pathologic anatomy*)
Meranze, David R., Mt. Sinai Hospital (*Clinical pathology and pathologic anatomy*)
Moon, Virgil H., Jefferson Medical College (*Clinical pathology and pathologic anatomy*)
Morgan, David R., Jefferson Medical College (*Clinical pathology and pathologic anatomy*)
Peale, Augustin R., Temple University School of Medicine (*Clinical pathology and pathologic anatomy*)
Pietroluongo, Anthony L., Temple University School of Medicine (*Pathologic anatomy*)
Rees, William T., St. Mary's Hospital (*Clinical pathology and pathologic anatomy*)
Reimann, Stanley P., 703 W. Phil-Ellena St. (*Clinical pathology and pathologic anatomy*)
Richardson, Russell, 320 S. 16th St. (*Clinical pathology and pathologic anatomy*)
Riggs, Helena, Phildelphia General Hospital (*Neuropathology*)
Rose, S. Brandt, Philadelphia General Hospital (*Clinical pathology*)
Sano, Machteld E., Temple University (*Pathologic anatomy*)
Spaeth, William L., 909 Unruh Ave. (*Clinical pathology*)
Stasney, Joseph, Jefferson Medical College (*Clinical pathology and pathologic anatomy*)
Thomas, Wilbur C., Temple University School of Medicine (*Pathologic anatomy*)
Tocantins, Leandro M., 1025 Walnut St. (*Clinical pathology*)
Waltz, Arthur D., Delaware County Hospital (*Clinical pathology and pathologic anatomy*)
Weiss, Charles, Jewish Hospital (*Clinical pathology*)
Zeckwer, Isolde R., University of Pennsylvania Medical School (*Pathologic anatomy*)
Zeidman, Irving, University of Pennsylvania Medical School (*Pathologic anatomy*)

PHILIPSBURG

Taylor, Frances M., 200 Hickory St. (*Clinical pathology and pathologic anatomy*)

PITCAIRN

Bosse, Milton D., 502 Broadway (*Clinical pathology and pathologic anatomy*)

PITTSBURGH

Ackerman, Milton, Aspinwall Veterans Administration Hospital (*Pathologic anatomy*)
Beauregard, John F., Elizabeth Steel Magee Hospital (*Pathologic anatomy*)
Bracken, Mark M., Pride & Locust Sts., Mercy Hospital (*Pathologic anatomy*)
Bruecken, Albert J., St. Francis Hospital (*Clinical pathology and pathologic anatomy*)
Burt, Robert C., Singer Laboratory, Allegheny General Hospital (*Clinical pathology and pathologic anatomy*)
Cohen, Mortimer, University of Pittsburg School of Medicine (*Clinical pathology and pathologic anatomy*)
Fetterman, George H., St. Margaret Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Graham, Allen, 4200 Friendship (*Pathologic anatomy*)
Grauer, Robert C., Allegheny General Hospital (*Clinical pathology and pathologic anatomy*)
Gross, Paul, 2117 Garson St. (*Clinical pathology and pathologic anatomy*)
Hamilton, Robert C., St. Francis Hospital (*Clinical pathology and pathologic anatomy*)
Haythorn, Samuel R., 320 E. North Ave. (*Pathologic anatomy*)
Heller, E. L., University of Pittsburgh School of Medicine (*Pathologic anatomy*)
Helmbold, Theodore R., South Side Hospital (*Clinical pathology and pathologic anatomy*)
Joyce, Francis W., 4001 California Ave. (*Clinical pathology and pathologic anatomy*)
Lacy, George R., Pittsburgh University School of Medicine (*Clinical pathology and pathologic anatomy*)
Mellon, Ralph R., Western Pennsylvania Hospital (*Clinical pathology*)

Menten, Maud L., University of Pittsburgh School of Medicine (*Clinical pathology and pathologic anatomy*)
Permar, Howard H., Mercy Hospital (*Clinical pathology and pathologic anatomy*)
Sherman, Frank E., Jr., 3500 5th Ave. (*Clinical pathology and pathologic anatomy*)
Siebert, Frank T., Shadyside Hospital (*Pathologic anatomy*)
Simon, Richard J., 1400-1430 Locust St. (*Pathologic anatomy*)
Stock, Aaron, 125 DeSota St. (*Clinical pathology and pathologic anatomy*)
Troll, Mary Maher, Pittsburgh Hospital (*Pathologic anatomy*)
Yardumian, Kriker Y., Montefiore Hospital (*Clinical pathology and pathologic anatomy*)

PHOENIXVILLE

Gentzkow, Oleon J., Lt. Col., Valley Forge General Hospital (*Clinical pathology and pathologic anatomy*)

READING

Funk, Erwin D., Reading Hospital (*Clinical pathology and pathologic anatomy*)
Jennings, William P., 1204 Oak Lane (*Clinical pathology and pathologic anatomy*)

SAYRE

DeWan, Charles H., Robert Packer Hospital (*Clinical pathology*)

SCRANTON

Clark, George A., 222 Cornell Bldg. (*Pathologic anatomy*)

SEWICKLEY

Unger, John, Sewickley Valley Hospital (*Clinical pathology*)

SHARON

Gillette, Perry C., C. H. Buhl Hospital (*Pathologic anatomy*)
LoCricchio, John L., 999 E. State Street (*Clinical pathology and pathologic anatomy*)

SHENANDOAH

Hobbs, Robert E., 28 E. Center (*Clinical pathology and pathologic anatomy*)

UNIONTOWN

Tolstoi, George, Uniontown Hospital Association (*Pathologic anatomy*)

UPPER DERBY

Kennedy, Patrick J., 6910 Market St. (*Clinical pathology and pathologic anatomy*)

WASHINGTON

Ramsey, G. W., Washington General Hospital (*Clinical pathology and pathologic anatomy*)

WAYNE

Porter, Mary McKee, 103 Walnut Ave. (*Clinical pathology*)

WEST CHESTER

Rothrock, Henry A., Jr. Chester County Hospital (*Clinical pathology and pathologic anatomy*)

WILKES-BARRE

Drapiewski, John F., Mercy Hospital (*Clinical pathology and pathologic anatomy*)
Tuthill, C. R., Wilkes-Barre General Hospital (*Pathologic anatomy*)

WILKINSBURG

Vandegrift, Earl, Columbia Hospital (*Clinical pathology and pathologic anatomy*)

WILLIAMSPORT

Colvin, Merle G., Williamsport Hospital (*Clinical pathology and pathologic anatomy*)

WILLOW GROVE

Love, John W. P., Major, MC, York Road & Maplewood Ave. (*Clinical pathology and pathologic anatomy*)

WYNNEWOOD

Norris, Robert F., 513 Wynnewood Ave. (*Clinical pathology and pathologic anatomy*)

YORK

Pusch, Lewis C., York Hospital (*Clinical pathology and pathologic anatomy*)

RHODE ISLAND

HOWARD

Goodman, Louis, Box 73 (*Pathologic anatomy*)

PROVIDENCE

Williams, Robert J., 593 Eddy St. (*Pathologic anatomy*)

SOUTH CAROLINA

CHARLESTON

Johnson, Francis B., State of South Carolina Medical College (*Clinical pathology*)
Lynch, Kenneth M., 16 Lucas St. (*Clinical pathology and pathologic anatomy*)
Pratt-Thomas, H. R., South Carolina Medical School (*Pathologic anatomy*)

COLUMBIA

Plowden, Henry H., 2020 Hampton St. (*Pathologic anatomy*)

FLORENCE

McMeans, Joseph W., McLeod Infirmary (*Clinical pathology and pathologic anatomy*)

SPARTANBURG

McGrew, Donald, Spartanburg General Hospital (*Clinical pathology and pathologic anatomy*)

SOUTH DAKOTA

MITCHELL

Moran, C. S., 119 E. Third Ave. (*Pathologic anatomy*)

RAPID CITY

Geib, Wayne A., St. Johns Hospital (*Clinical pathology and pathologic anatomy*)

SIOUX FALLS

Clarke, James Y., Sioux Valley Hospital, Euclid Ave. & 19th St. (*Pathologic anatomy*)
Pekelis, Emanuel, 7th Ave. & 21st St. (*Pathologic anatomy*)

VERMILLION

Ferguson, Ralph L., University of South Dakota (*Pathologic anatomy*)

TENNESSEE

CHATTANOOGA

Adams, John W., Jr., Erlanger Hospital (*Clinical pathology*)

KNOXVILLE

Mahon, George S., St. Mary's Hospital (*Clinical pathology and pathologic anatomy*)

MEMPHIS

Diggs, L. W., 42 North Dunlap (Unit 5) (*Clinical pathology*)
Dubin, Isadore N , Institute of Pathology, University of Tennessee (*Pathologic anatomy*)
Eberson, Frederick, Kennedy Veterans Administration Hospital (*Clinical pathology*)
Golden, Alfred, Institute of Pathology, University of Tennessee (*Pathologic anatomy*)
Gose, A. C., Gose Clinic & Laboratory (*Pathologic anatomy*)
Greiner, Daniel J., Kennedy Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Jones, Russell S., Institute of Pathology, University of Tennessee (*Pathologic anatomy*)
Leake, Nolan E., Baptist Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Michelson, Israel D., University of Tennessee Pathology Institute (*Clinical pathology and pathologic anatomy*)
Moss, Thomas C., 264 Jackson Ave. (*Clinical pathology and pathologic anatomy*)
Schmeisser, Harry C., University of Tennessee Pathology Institute (*Clinical pathology and pathologic anatomy*)
Sprunt, Douglas H., University of Tennessee (*Clinical pathology and pathologic anatomy*)
Tribby, William W., Methodist Hospital (*Clinical pathology and pathologic anatomy*)
Trumbull, Merlin L., 899 Madison Ave. (*Pathologic anatomy*)

MOUNTAIN HOME

Wilson, M. W., Box 1276, Veterans Administration (*Pathologic anatomy*)

NASHVILLE

Auerbach, Stewart H., Thayer Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Cuff, J. R., 2718 Scovel St. (*Pathologic anatomy*)
Dawson, James R , Vanderbilt University (*Pathologic anatomy*)
DeMonbreun, William A., Nashville General Hospital (*Clinical pathology and pathologic anatomy*)
Gotwald, David K., St. Thomas Hospital (*Clinical pathology and pathologic anatomy*)
Spitz, Herman, 706 Church St. (*Clinical pathology*)

TEXAS

AUSTIN

Bohls, Sidney W., 803 E. 32nd St (*Clinical pathology*)
Dunlop, Josephine, 116 W. 15th St. (*Clinical pathology and pathologic anatomy*)

BEAUMONT

Furey, Ellen D., 925 San Jacinto Bldg. (*Clinical pathology and pathologic anatomy*)

CORPUS CHRISTI

Pilcher, John F., 320 Medical Professional Bldg. (*Clinical pathology and pathologic anatomy*)

DALLAS

Bell, Marvin DeWitt, 1109 Medical Arts Bldg. (*Clinical pathology*)
Cairns, A. B., 2006 Hedgerow (*Clinical pathology and pathologic anatomy*)
Caldwell, Janet A., 3929 Potomac Ave. (*Clinical pathology and pathologic anatomy*)
Carter, Charles F., Medical Arts Bldg. (*Clinical pathology*)
Furth, Jacob, Veterans Administration Hospital (*Pathologic anatomy*)
Gill, Atticus J., 2211 Oak Lawn (*Pathologic anatomy*)
Goforth, John L., 3121 Dryan St (*Clinical pathology and pathologic anatomy*)
Hill, Joseph M., Baylor Hospital (*Clinical pathology and pathologic anatomy*)
Muirhead, E. E., Baylor Hospital (*Clinical pathology and pathologic anatomy*)

EL PASO

Hart, Maynard S., 310 Banner Bldg. (*Clinical pathology*)
Hause, Welland A., Major, Beaumont General Hospital (*Clinical pathology and pathologic anatomy*)

FORT SAM HOUSTON

Bayliss, Milward, Brooke General Hospital (*Clinical pathology*)
De Coursey, Elbert, Brooke General Hospital (*Pathologic anatomy*)
Hersohn, William W., 10th Medical Laboratory (*Clinical pathology*)
Thomas, A. R., Brooke General Hospital (*Pathologic anatomy*)
Van Auken, Howard A., Brooke General Hospital (*Clinical pathology*)

FORT WORTH

Ashworth, Charles T., 604 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Andujar, John J., 1300 W. Cannon Ave. (*Clinical pathology*)
Owen, May, Box 1719 (*Clinical pathology and pathologic anatomy*)
Terrell, Truman C., 602 W. Tenth St. (*Clinical pathology and pathologic anatomy*)

GALVESTON

Brindley, Paul, University of Texas School of Medicine (*Pathologic anatomy*)
Neidhardt, H. W., 925 Avenue B. (*Pathologic anatomy*)
Rigdon, Raymond H., University of Texas (*Pathologic anatomy*)
Schofield, Norman D., University of Texas (*Pathologic anatomy*)
Townsend, Frank, 220 E. Harrison (*Pathologic anatomy*)

HOUSTON

Braden, Albert H., 1704 Crawford St. (*Clinical pathology and pathologic anatomy*)
Chappell, R. H., Memorial Hospital (*Clinical pathology and pathologic anatomy*)
Coulter, W. W., Sr., 422 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Davenport, Harbert, Baylor University College of Medicine (*Pathologic anatomy*)
Fowler, Kenneth, 1603 North Blvd. (*Clinical pathology and pathologic anatomy*)
Keiller, Violet H., 4218 Austin St. (*Clinical pathology and pathologic anatomy*)
Marcuse, Peter M., Baylor University (*Clinical pathology and pathologic anatomy*)
Powell, Elizabeth D., Baylor University (*Pathologic anatomy*)
Russell, William O., 2310 Baldwin St. (*Clinical pathology and pathologic anatomy*)
Sanders, Charles B., 1215 Walker Ave. (*Clinical pathology and pathologic anatomy*)
Wallace, Stuart A., Baylor University College of Medicine (*Clinical pathology and pathologic anatomy*)
Wells, Benjamin B., 2310 Baldwin St. (*Clinical pathology*)

LEGION

Hentel, William, Veterans Administration Hospital (*Pathologic anatomy*)

McKINNEY

Cromartie, William J., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Wollenman, O. J., Jr., Ashburn Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

SAN ANGELO

Hershberger, Lloyd, Shannon Memorial Hospital (*Clinical pathology and pathologic anatomy*)

SAN ANTONIO

Moore, John M., 818 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Schattenberg, Herbert J., 222 Medical Arts Bldg. (*Clinical pathology and pathologic anatomy*)
Severance, Alvin O., 205 Camden St. (*Clinical pathology and pathologic anatomy*)
Stout, Beecher F., 705 E. Houston St. (*Clinical pathology and pathologic anatomy*)

TEMPLE

Liber, A. F., McCloskey Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Phillips, Charles, 1304 North Third St. (*Pathologic anatomy*)
Powell, W. N., Scott & White Clinic (*Clinical pathology and pathologic anatomy*)
Rice Walter Gowaus, Kings Daughters Clinic (*Pathologic anatomy*)

WICHITA FALLS

Pasternack, Joseph G., Box 2016 (*Clinical pathology and pathologic anatomy*)

UTAH

OGDEN

Ellis, Ralph C., St. Benedicts Hospital (*Clinical pathology and pathologic anatomy*)
Zeman, Erwin D., 2440 Harrison Blvd. (*Clinical pathology and pathologic anatomy*)

SALT LAKE CITY

Carlquist, J. H., 147 E. Second St. (*Pathologic anatomy*)
Gunn, F. D., University of Utah School of Medicine (*Clinical pathology and pathologic anatomy*)
McNeil, C., Holy Cross Hospital (*Clinical pathology and pathologic anatomy*)
Ogilvie, Orin A., 916 Military Dr. (*Clinical pathology and pathologic anatomy*)
Sheehy, John J., University of Utah School of Medicine (*Clinical pathology and pathologic anatomy*)

VERMONT

BENNINGTON

Flory, Curtis M., Henry Putnam Memorial Hospital (*Pathologic anatomy*)

BURLINGTON

Buttles, Ernest H., 457 S. Willard St. (*Clinical pathology and pathologic anatomy*)
Ferguson, John A., Medical College, University of Vermont (*Clinical pathology and pathologic anatomy*)
Morrione, Thomas G., Medical College, University of Vermont (*Clinical pathology and pathologic anatomy*)
Pearson, Bjarne, Medical College, University of Vermont (*Pathologic anatomy*)
Stark, Ernest, Medical College, University of Vermont (*Pathologic anatomy*)
Woll, Ephraim, Medical College, University of Vermont (*Pathologic anatomy*)

RUTLAND

McBride, W. P., Rutland Hospital (*Clinical pathology*)

VIRGINIA

ALEXANDRIA

Dowling, George B., 44 Woodmont Road, Belle Haven (*Clinical pathology*)
Kernodle, J. D., Alexandria Hospital (*Clinical pathology and pathologic anatomy*)

ARLINGTON

Sloan, Ruell A., 1805 S. Pollard St. (*Pathologic anatomy*)

CHARLOTTESVILLE

Bray, William E., Box 1063, University (*Clinical pathology and pathologic anatomy*)
Parker, Francis P., University of Virginia (*Clinical pathology and pathologic anatomy*)

CLIFTON FORGE

Dreyfuss, Martin, Chesapeake & Ohio Hospital (*Clinical pathology and pathologic anatomy*)

DANVILLE

Moran, T. J., The Memorial Hospital (*Clinical pathology and pathologic anatomy*)

KECOUGHTAN

Baron, Edgar, Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

LYNCHBURG

Bouton, S. Miles, Jr., Lynchburg General Hosp. (*Clinical pathology and pathologic anatomy*)

NEWPORT NEWS

Beecroft, Morris B., Buxton Clinic (*Clinical pathology and pathologic anatomy*)

NORFOLK

Motyca, Lawrence J., 7419 Muirfield Rd. (*Clinical pathology and pathologic anatomy*)
Strauss, Arnold F., De Paul Hospital (*Pathologic anatomy*)

PORTSMOUTH

Wellbreck, W. L. A., U. S. Naval Hospital (*Clinical pathology and pathologic anatomy*)

RICHMOND

Apperly, Frank L., Medical College of Virginia (*Clinical pathology and pathologic anatomy*)
Beck, Regena C., Stuart Circle Hospital (*Clinical pathology and pathologic anatomy*)
Butcher, James, Medical College of Virginia (*Clinical pathology and pathologic anatomy*)
Kriz, Joseph, Veterans Administration Hospital (*Clinical pathology*)
Russi, Simon, Veterans Administration Hospital (*Pathologic anatomy*)
Sahyoun, Philip, Medical College of Virginia (*Pathologic anatomy*)
Scherer, John H., 820 W. Franklin St. (*Clinical pathology and pathologic anatomy*)
Scotti, Thomas M., Medical College of Virginia (*Clinical pathology and pathologic anatomy*)
Williams, George Z., Medical College of Virginia (*Pathologic anatomy*)

ROANOKE

Porro, Francis W., Roanoke Hospital (*Clinical pathology and pathologic anatomy*)

WINCHESTER

Murphy, George H., Winchester Memorial Hospital (*Pathologic anatomy*)

WASHINGTON

BREMERTON

Hall, William W., U. S. Naval Dispensary (*Clinical pathology and pathologic anatomy*)

EVERETT

Bitar, Emmanuel, General Hospital of Everett (*Pathologic anatomy*)

SEATTLE

Cefalu, Victor, 509 Olive St. (*Clinical pathology and pathologic anatomy*)
Chipps, H. D., University of Washington School of Medicine (*Clinical pathology and pathologic anatomy*)
Edmonds, Henry W., 909 University St. (*Pathologic anatomy*)
Jensen, Clyde R., 1305 Fourth Ave. (*Pathologic anatomy*)
Lippincott, S. W., University of Washington (*Pathologic anatomy*)
Lund, Paul K., Swedish Hospital (*Pathologic anatomy*)
Mason, David G., Providence Hospital (*Clinical pathology and pathologic anatomy*)
Nickson, Delbert H., 803 Summit Ave. (*Clinical pathology and pathologic anatomy*)
Ricker, Walter A., Jr., University of Washington School of Medicine (*Pathologic anatomy and clinical pathology*)
Tooley, George E., U. S. Marine Hospital (*Pathologic anatomy*)

SPOKANE

Christianson, Oscar O., St. Luke's Hospital (*Pathologic anatomy*)
Patton, Frank R., 264 Paulsen Bldg. (*Clinical pathology and pathologic anatomy*)
Patton, Matthew M., 264 Paulsen Bldg. (*Clinical pathology and pathologic anatomy*)

TACOMA

Larson, Charles P., Tacoma General Hospital (*Clinical pathology and pathologic anatomy*)
McColl, Charles R., P.O. Box 1625 (*Clinical pathology and pathologic anatomy*)
Terry, Benjamin T., 1001 N. Yakima Ave. (*Clinical pathology and pathologic anatomy*)

VANCOUVER

Van Brown, David, Veterans Administration (*Pathologic anatomy*)

WALLA WALLA

Foote, Edward L , 7 S. Touchet St. (*Pathologic anatomy*)

YAKIMA

Shirey, Ralph W., 102 S. Ninth Ave. (*Clinical pathology and pathologic anatomy*)

WEST VIRGINIA

BECKLEY

Mosteller, Ralph, Beckley Hospital (*Clinical pathology and pathologic anatomy*)

CHARLESTON

Newman, Benjamin, Kanawha Valley Hospital (*Clinical pathology and pathologic anatomy*)
Putschar, Walter G. J., Charleston General Hospital (*Clinical pathology and pathologic anatomy*)

FAIRMONT

Nolan, Lewis Earle, Fairmont General Hospital (*Clinical pathology and pathologic anatomy*)

HUNTINGTON

Hodges, Frank C., 955 Fourth Ave. (*Clinical pathology and pathologic anatomy*)
Levy, Fritz, Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)
Werthammer, Siegfried, 2008 N. Englewood Rd. (*Pathologic anatomy*)

MARTINSBURG

Greene, Ralph C., Newton W. Baker Veterans Administration Hospital (*Pathologic anatomy*)

MORGANTOWN

Fenton, Clement C., 222 Wilson Ave. (*Clinical pathology and pathologic anatomy*)
Hobbs, Milford, West Virginia University (*Pathologic anatomy*)

PARKERSBURG

Kanner, Oscar, St. Joseph's Hospital (*Clinical pathology*)

PHILIPPI

Myers, E. E., Myers Clinic Hospital (*Clinical pathology*)

WHEELING

Little, H. G., 2000 Eoff St. (*Clinical pathology and pathologic anatomy*)

WISCONSIN

GREEN BAY

Ford, John L., St. Vincent's Hospital (*Clinical pathology and pathologic anatomy*)
Heitzman, Holly H., 403 S. Webster St. (*Pathologic anatomy*)

LACROSSE

Bach, Amil C., 207 Fifth Ave S. (*Pathologic anatomy*)

MADISON

Angevine, D. Murray, University of Wisconsin (*Pathologic anatomy*)
Bleyer, Leo F., 720 S. Brooks (*Clinical pathology and pathologic anatomy*)
Jaeschke, W. H., State of Wisconsin Hospital (*Clinical pathology and pathologic anatomy*)
McGary, Lester, 925 Mound St. (*Clinical pathology and pathologic anatomy*)
Schafer, Etheldred L., Jackson Clinic, 16 South Henry Street (*Pathologic anatomy*)
Stovall, William D., University of Wisconsin (*Clinical pathology and pathologic anatomy*)

MARSHFIELD

Miale, J. B., Marshfield Clinic (*Clinical pathology and pathologic anatomy*)

MILWAUKEE

Anderson, W. A. D., Marquette University School of Medicine (*Clinical pathology and pathologic anatomy*)
Barta, Edward F., 425 E. Wisconsin Ave. (*Clinical pathology and pathologic anatomy*)
Birge, Edward A., 2200 West Kilbourn St. (*Clinical pathology and pathologic anatomy*)
Enzer, Norbert, 425 E. Wisconsin Ave. (*Clinical pathology and pathologic anatomy*)
Grill, John C., Marquette University Medical School (*Clinical pathology and pathologic anatomy*)
Hansmann, George H., 831 N. Prospect Ave. (*Clinical pathology and pathologic anatomy*)
Haukohl, Robert S., Marquette University School of Medicine (*Pathologic anatomy*)
Pessin, Samuel B., St. Mary's Hospital, 2320 N. Lake Drive (*Clinical pathology and pathologic anatomy*)

Ritchie, Gorton, 3321 N. Maryland Ave. (*Clinical pathology and pathologic anatomy*)
Rosenzweig, Maurice, 908 N. 12th St. (*Clinical pathology and pathologic anatomy*)
Schuster, Myron, Milwaukee County Hospital (*Clinical pathology and pathologic anatomy*)
Tharinger, Edward L., 231 W. Wisconsin Ave. (*Clinical pathology and pathologic anatomy*)
Van Hecke, Leander J., 231 W. Wisconsin Ave. (*Clinical pathology and pathologic anatomy*)

NEENAH

Strauser, E. R., 316 Carolina St. (*Clinical pathology and pathologic anatomy*)

OCONTO

Rogers, Raymond J., Box 88 (*Pathologic anatomy*)

WAUWATOSA

Kuzma, Joseph F., 8700 W. Wisconsin Ave. (*Clinical pathology and pathologic anatomy*)

WOOD

Lubitz, Joseph M., Veterans Administration Hospital (*Clinical pathology and pathologic anatomy*)

WYOMING

CHEYENNE

Zuckerman, Samuel S., 1606 Capitol Avenue (*Pathologic anatomy*)

CANAL ZONE

PANAMA

Clark, Herbert Charles, 68 Avenida Justo Arosemena, P.O. Apratado 1252, Panama, R. de P. (*Clinical pathology and pathologic anatomy*)

HAWAII

HONOLULU

Fennel, Eric A., 881 Young St. (*Clinical pathology and pathologic anatomy*)
Price, Aaron Sumner, Queen's Hospital (*Clinical pathology and pathologic anatomy*)
Tilden, Irvin L., The Clinic, 881 South Hotel St. (*Pathologic anatomy*)

PHILIPPINE ISLANDS

MANILA

Sta Cruz, Juan E., St. Luke's Hospital (*Clinical pathology and pathologic anatomy*)

PUERTO RICO

PONCE

Costa-Mandry, O., Clinica Dr. Pila (*Clinical pathology*)

SAN JUAN

Koppisch, Enrique, Schoo lof Tropical Medicine (*Clinical pathology and pathologic anatomy*)
Reyes, Felix M., Veterans Administration Patricio Hospital (*Pathologic anatomy*)

DOMINION OF CANADA

ALBERTA

CALGARY

McLatchie, Lola Dell, Calgary Clinical Laboratory (*Clinical pathology and pathologic anatomy*)

EDMONTON

Hanson, Samuel, Edmonton General Hospital (*Clinical pathology and pathologic anatomy*)

BRITISH COLUMBIA

VICTORIA

McCurdy, Gordon Alexander, Royal Jubilee Hospital (*Clinical pathology and pathologic anatomy*)

MANITOBA

ST. JAMES

Williams, Thomas H., 257 Duffield St., Dear Lodge Hospital (D.V.A.) (*Clinical pathology and pathologic anatomy*)

WINNIPEG

Nicholson, Daniel, University of Manitoba Faculty of Medicine (*Clinical pathology and pathologic anatomy*)

NEW BRUNSWICK

ST. JOHNS

Branch, E. Arnold G., Bureau of Laboratories, General Hospital (*Clinical pathology and pathologic anatomy*)
Mackeen, Robert A. H., General Hospital (*Clinical pathology and pathologic anatomy*)

NOVA SCOTIA

HALIFAX

Smith, Ralph P., Dalhousie University, Pathology Institute, Morris St. (*Clinical pathology and pathologic anatomy*)

MEADOWVILLE

Ross, Elizabeth, Meadowville R. R. #1 (*Clinical pathology and pathologic anatomy*)

ONTARIO

HAMILTON

Deadman, William J., Hamilton General Hospital (*Clinical pathology and pathologic anatomy*)

TORONTO

Wyatt, J. Poyner, Toronto East General Hospital (*Pathologic anatomy*)

QUEBEC

MONTREAL

Simon, Morris A., 3755 St. Catherine Road (*Clinical pathology and pathologic anatomy*)
Waugh, Theodore R., 3755 University St. (*Clinical pathology and pathologic anatomy*)
Wiglesworth, Frederick W., Children's Memorial Hospital (*Pathologic anatomy*)

QUEBEC

Berger, Louis, Laval University Pathology Institute (*Clinical pathology and pathologic anatomy*

CHINA

CHENGTU SZECHUAN

Iverson, Lalla, West China Union (*Pathologic anatomy*)

GERMANY

STUTTGART

Reichle, Herbert S., St. Katherine's Hospital (*Clinical pathology and pathologic anatomy*)

INDIA

BOMBAY

Gharpure, Vithal Vishvanath, Clinical Laboratory, 489 Adenwalla Road Matunga (*Pathologic anatomy*)

NEW ZEALAND

DUNEDIN

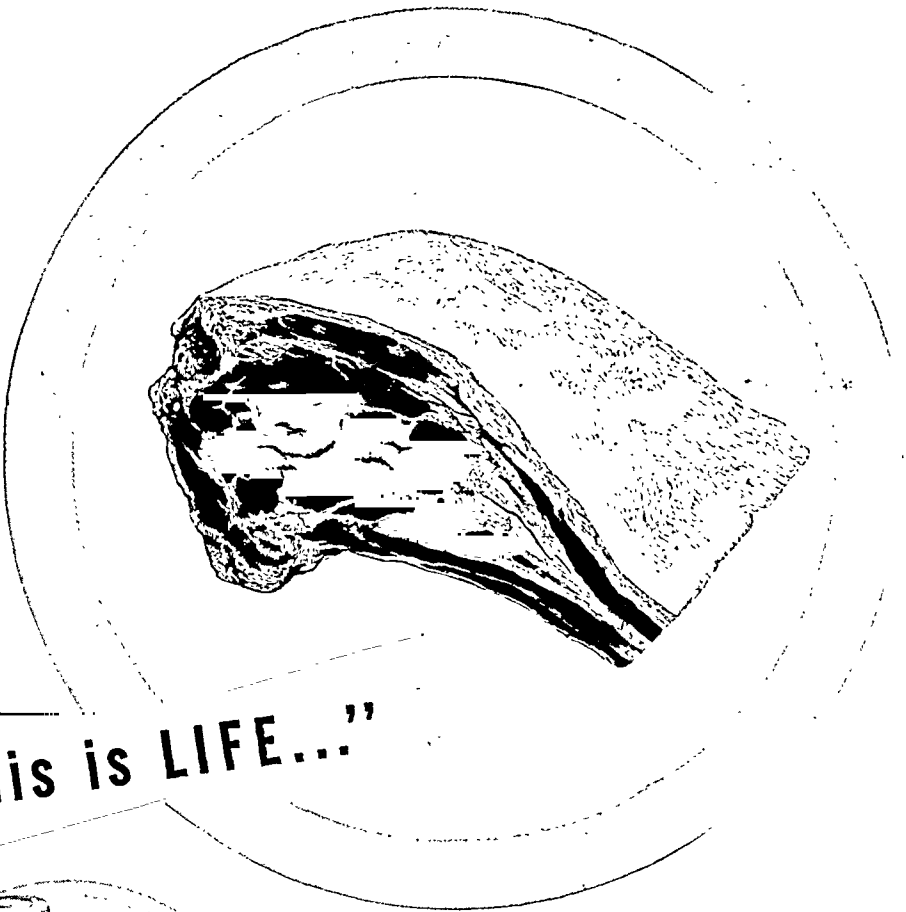
Alexander, W. Stewart, King St. (*Pathologic anatomy*)

SOUTH AMERICA

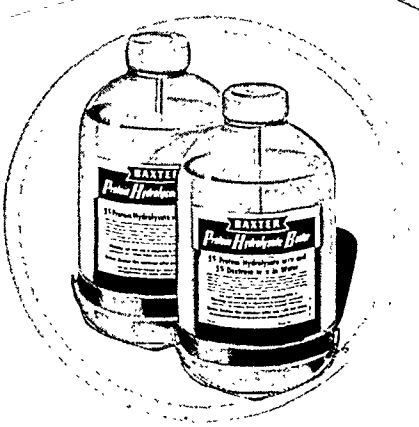
COSTA RICA

SAN JOSE

Fallas-Diaz, Marcial, Hospital San Juan de Dioz (*Pathologic anatomy*)



"This is LIFE..."



Protein Hydrolysate

BAXTER

Available with or without Dextrose

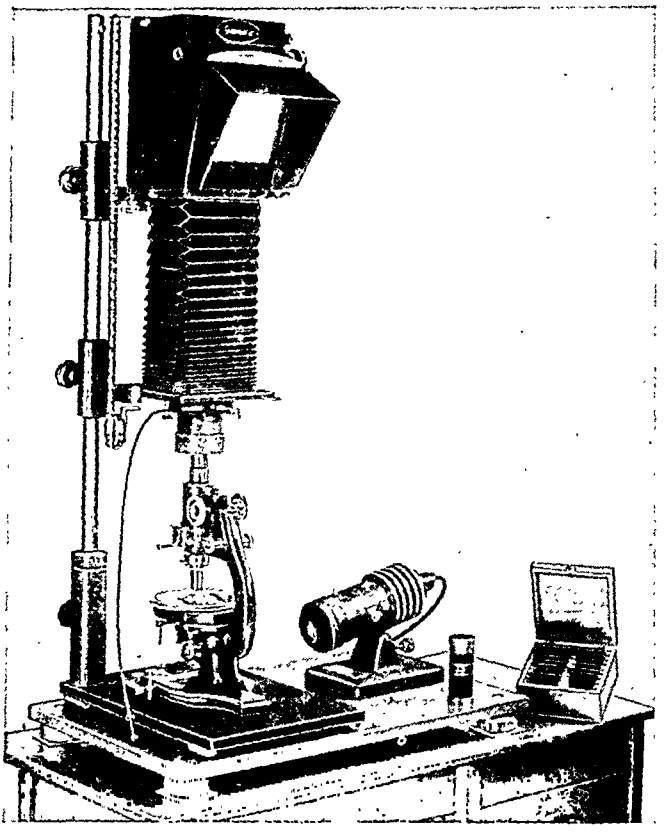
Protein is essential in the diet. It is life itself. But people cannot always have it in the palatable form of red meat. For example, a surgical patient faces the shock of tissue destruction, blood loss, loss of body protein. Before and after surgery, Protein Hydrolysate Baxter materially helps to meet the protein needs of the surgical patient. This new achievement of Baxter research is a beef product . . . prepared from bovine plasma . . . enzymatically digested. Autoclaved, proven sterile and non-pyrogenic, it is conspicuously free from reactions. Full information is available on request.

Baxter Laboratories, Inc.
Morton Grove, Ill.

Distributed and available only in the 37 states east of the Rockies through—

American Hospital Supply Corporation • General Offices, Evanston, Illinois

(In writing to advertisers, please mention the Journal—it helps.)



GAMMA
"MICROFLEX"
with
ZIRCON ARC LAMP

THE MOST MODERN CAMERA
For Photomicrography
LOW POWER and
MACRO Photography

WRITE FOR FOLDER G22

to

GAMMA INSTRUMENT CO., INC.

P.O. BOX 532

GREAT NECK, L. I., N. Y.

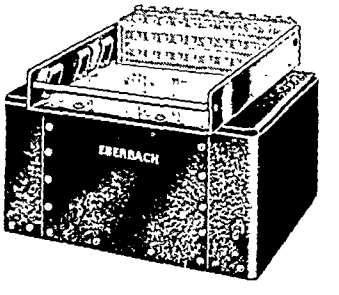
Golden Hamsters

Hamsters are ideal laboratory animals that are economical and dependable to use. They are sturdy healthy animals that can be easily fed, housed, and handled in your laboratory. No objectionable odor or noise.

Our years of experience assure you of dependable service. An unusually liberal guarantee protects you. We will be pleased to quote you on your requirements at any time.

HUDSON HAMSTERY

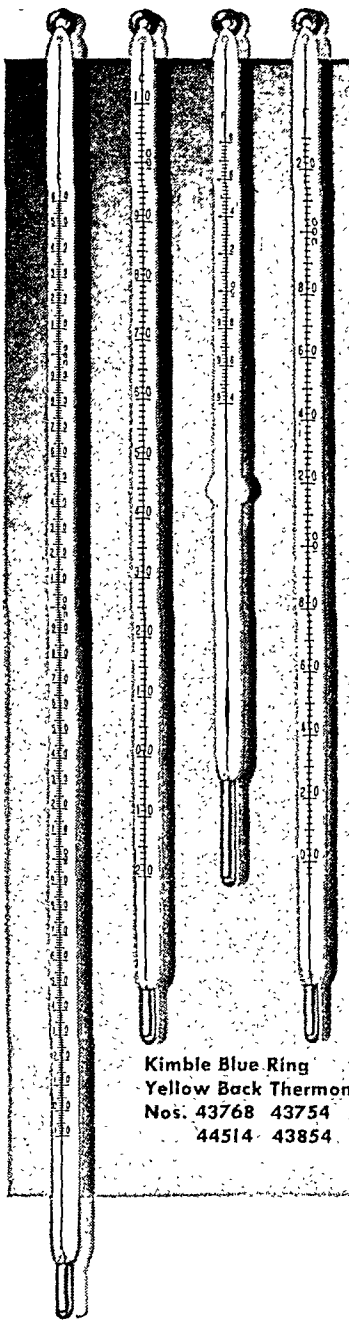
NEWFOUNDLAND 4, N. J.



Eberbach
KAHN SHAKERS

These shakers agitate at 280 oscillations per minute through a 1½ inch stroke as specified by Dr. Reuben L. Kahn of the University of Michigan. Both 4 and 6 rack models are made. Complete information available on request.

Eberbach & Son Company
ANN ARBOR, MICH.
LABORATORY APPARATUS & SUPPLIES
ESTABLISHED 1849



Kimble Blue Ring
Yellow Back Thermometers,
Nos. 43768 43754
44514 43854

Retested . . .
Accurate . . .
Readable . . .

Kimble Blue Ring Yellow Backs

are here!

KIMBLE GLASS . . . foremost maker of precision laboratory glassware . . . proudly presents this outstanding new line of retested, accurate, easily read thermometers. Every item in this new line is *retested* to insure its accuracy.

Precise! There is a Kimble Blue Ring Yellow Back Thermometer for each scientific specification—ASTM and other special-purpose thermometers—National Bureau of Standards thermometer specifications . . . can be N.B.S. Certified if required.

High visibility! The yellow back behind the column and the *new style horizontal* numbering insure speediest and most accurate reading . . . save your time and minimize possibility of error.

Look for the blue ring on top that instantly identifies genuine Kimble Yellow Backs.

If your laboratory supply dealer does not yet have Kimble Blue Ring Yellow Back Thermometers, write us for the names of the nearest dealers who have them.

KIMBLE GLASS TOLEDO 1, OHIO

Division of Owens-Illinois Glass Company

© 1949—O.I.G. Co.

for real help in understanding
the fundamentals of a difficult subject

TOPICS *in* PHYSICAL CHEMISTRY

By

W. MANSFIELD CLARK, Ph.D., Sc.D.

DeLamar Professor of Physiological Chemistry, Johns Hopkins University School of Medicine

Certain principles of chemistry are universally recognized. Students of this science, wherever trained, should possess much knowledge in common. However, Dr. Clark in his years of teaching biochemistry has found discrepancies in the training of his students due to differences in teaching methods in pre-medical schools, and to the students themselves.

The material in this book ranges over a wide field. The chemical balance and measurements with volumetric apparatus are described as well as more abstruse subjects. In every case, Dr. Clark's experience has shown that such review material is needed. He has chosen to discuss those topics with which students have shown they most need help.

For the student, the book supplies the supplementary reading needed in courses in biochemistry, and for the practitioner it furnishes a simple and ready means of reviewing principles for a better understanding of application.

753 pages 300 illustrations $10.00

THE WILLIAMS & WILKINS COMPANY

Mt. Royal and Guilford Aves. *Baltimore 2, Md.*

TESTING REAGENTS
for
Hematology and Serology

Anti-A and Anti-B Serum
for Blood Grouping

Anti-Rh Serum
for determining presence of Rh factor

Sub-Groups of Anti-Rh Serum

Coombs' Serum
for antibody test

Anti-M and Anti-N Serum
for paternity tests

Absorbed B Serum
to differentiate between A_1 and 2_2

Human red cells for research work or controls

Sheep Cells

Complement

Amboceptor

Kline Antigen

Kahn Antigen

Mazzini Antigen

Kolmer Antigen

Positive Syphilitic serum
for control tests

P.S.A. (Paraffin Section Adhesive)

CERTIFIED BLOOD DONOR SERVICE

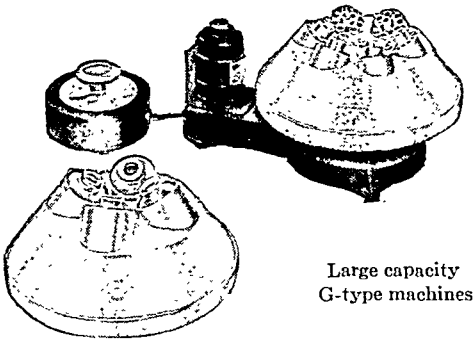
146-16 Hillside Avenue Jamaica 2, New York

(In writing to advertisers, please mention the Journal—it helps.)

SERVALL

ANGLE CENTRIFUGES

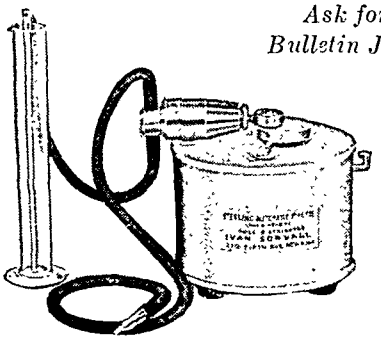
Ask for Bulletin J-21
with complete data on all models



Large capacity
G-type machines

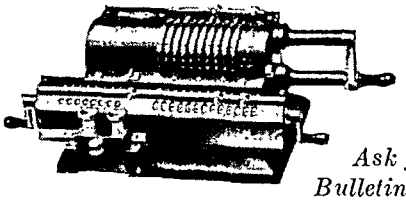
Type G/1, for 6 bottles of 250 cc each
18 tubes of 60 cc each
42 tubes of 15 cc each
78 tubes of 10 cc each
Type G/2, for 4 bottles of 500 cc each
Type G/3, for 10 tubes of 100 cc each

Ask for
Bulletin J-22



STERLING AUTOMATIC
AND
HAND PIPETTES

Dispense accurately, at a set speed, predetermined amounts of 0.1 cc to 10.0 cc.
Easy to operate—Time Saving



Ask for
Bulletin J-23

ORIGINAL—ODHNER

Portable Calculator for the Scientist
Many different models
Efficient - Sturdy - Low Priced

Manufacturers and Distributors:

IVAN SORVALL, Inc.

210 FIFTH AVE.-NEW YORK 10, N. Y.

Advertisers
in the Journal
Enjoy these advantages

- Only carefully selected advertisers are invited to use space. This assures the quality of the advertising and effectiveness of results.
- The Clinical Pathologist is the individual responsible for the selection and purchase of equipment and supplies. The advertiser's message reaches him directly.
- Readers appreciate the part that advertising revenue plays in making publication possible. This factor is an influence in their choice of where to buy.

Sixteenth Edition—

Stedman's Practical Medical Dictionary

Edited by NORMAN B. TAYLOR, M.D.
University of Toronto

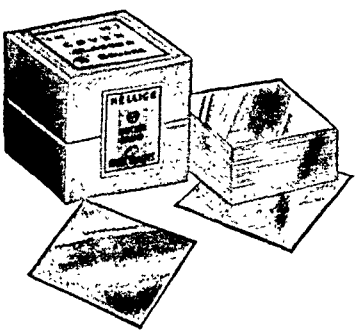
- ▶ Hundreds of new titles and subtitles added, hundreds rewritten, obsolete terms omitted.
- ▶ Continues to be the "last word" in the pronunciation, spelling, and meaning of medical terms.

1292 pages *23 plates* *$7.50*

SANS TACHE

THE WILLIAMS & WILKINS CO.
Mt. Royal and Guilford Aves. • Baltimore 2, Md.

HELLIGE
CHECKER BRAND
MICRO
COVER GLASSES
Again Available!



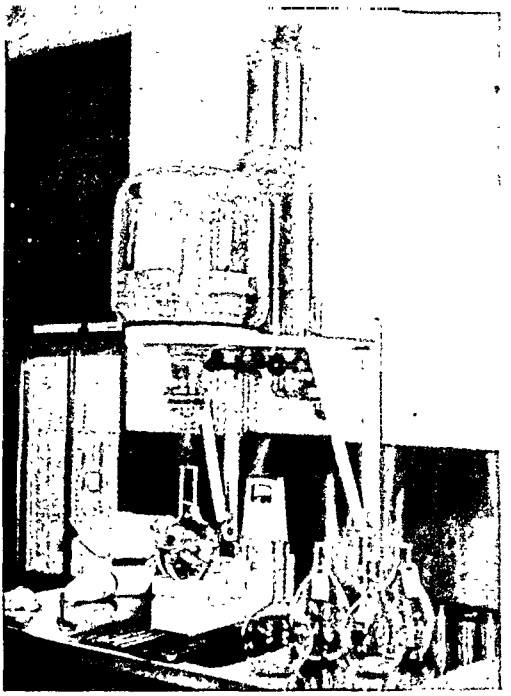
We have resumed the production of micro cover glasses, discontinued during the war because our efforts were directed primarily toward manufacturing for the U.S. Army and Navy Hellige specialties not obtainable elsewhere.

Now we are able to offer again the same surpassing quality which made Hellige Micro Cover Glasses the choice of the medical profession for more than half a century.

Write Today for our Attractive Prices

HELLIGE
INCORPORATED
3718 NORTHERN BLVD.
LONG ISLAND CITY 1, N.Y.

Castle
REFLUX STILL



Steam Heat Only

Produces distilled water free from pyrogen and harmful impurities

The Castle Reflux Still produces approximately 10 gallons of pyrogen-free distillate per hour, with a greater degree of purity than any other standard apparatus or process. It is completely automati . easy to clean . . . safe and dependable!

Pre-heating of the water in a specially designed puddle chamber drives off gases . . . precipitates lime salts . . . starts purification of the water even before it gets to the evaporating chamber. Deconcentrating tube with visible glass chamber prevents concentration of solids in evaporator. Vapor has 27-inch rise interrupted by multiple baffles which prevent contamination of condenser by foaming or splashing.

Operating on the Reflux Principle, the primary condenser returns one-quarter of the vapor . . . particularly the larger particles . . . to the evaporator. Only the finest vapor passes over to the secondary condenser producing the final distillate.

For further details, write for Bulletin D-2. Wilmot Castle Co., 1216 University Ave., Rochester 7, N. Y.

Write for Bulletin D-2

Castle **BACTERIOLOGICAL APPARATUS**

Shortage of Medical Technologists

Why do medical technologists leave the field?

In 1948, *357* female registered medical technologists were withdrawn from the Registry files because they were delinquent 3 years in payment of their renewal fees.

Their records are revealing:

At time of last renewal (1945)

Married	69%
Housewives	31%

How long had these people been registered before they left medical technology?

At the time of withdrawal (1948)

15 or more years	12%
10 to 15 years	33%
5 to 10 years	55%

These figures help to explain the shortage in this field, and why constant recruitment is necessary, if this condition is to be remedied.

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
American Society of Clinical Pathologists
Muncie, Indiana

THE
DIAGNOSIS
AND
TREATMENT
OF
BRONCHIAL ASTHMA

By Leslie N. Gay, M.D.

Foreword by Warfield T. Longcope, M.D.

- Presents the well-founded information of the present day concerning asthma and asthmatic symptoms, in addition to the conflicting theories and ideas about etiology and treatment.
- Emphasizes importance of complete history of patient before diagnosis.
- Outlines causes of asthmatic conditions.
- Describes examinations and tests used in diagnosis.
- Discusses complications and differential diagnosis.
- Explains treatments for various types of asthmatic complaints.
- Points out procedures of questionable value.

For the medical student, the internist or medical consultant, and the general practitioner

327 pp. 88 illus. $5.00

SANS TACHE

THE WILLIAMS & WILKINS CO.
Mt. Royal and Guilford Aves.
Baltimore 2, Md.

ANOTHER MOSBY BOOK!

CANCER of the Esophagus and Gastric Cardia

Edited by

GEORGE T. PACK, B.S., M.D.,

New York, N. Y.

Clinical Professor of Surgery, New York Medical College; Attending Surgeon, the Memorial Hospital for Cancer and Allied Diseases

192 Pages *Illustrated* *PRICE, $5.00*

The symposium which comprises this book originally appeared in the June, 1948 issue of SURGERY. Many requests were received to have it issued as a monograph, and as such it embodies the most recent and most notable achievements in the treatment of cancers of the esophagus.

CONTENTS

Introduction. George T. Pack, M.D., New York City.

The Roentgen Diagnosis of Cancer of the Cardian Region of the Stomach. Robert S. Sherman, M.D., New York City.

Preoperative Operative, and Postoperative Care in Esophageal Resections. Herbert C. Maier, M.D., New York City.

Cancer of the Cervical Esophagus. William L. Watson, M.D., New York City; and John L. Pool, M.D., New York City.

Progress in the Surgical Treatment of Carcinoma of the Esophagus and Upper Stomach. John H. Garlock, M.D., New York City.

Transthoracic Gastric Resection for Lesions of the Cardia of the Stomach and Middle and Lower Portions of the Esophagus. Frank S. Ashburn, M.D., Rochester, Minn.; John H. Payne, M.D., Cincinnati, Ohio; and O. Theron Clagett, M.D., Rochester, Minn.

Cardioesophageal Cancers Treated Via the Transthoracic and Transdiaphragmatic Route. Felix de Amesti, M.D., Santiago, Chili; Eliseo Otaiza, M.D., Santiago, Chili.

Subtotal Esophagectomy and Esophagogastrostomy for High Intrathoracic Esophageal Lesions. Michael E. DeBakey, M.D., New Orleans, Louisiana; Alton Ochsner, M.D., New Orleans, Louisiana.

Combined Left Abdominal and Right Thoracic Approach to Resection of Esophageal Neoplasms. Joseph E. Macmanus, M.D., Buffalo, New York.

The Treatment of Carcinoma of the Esophagus and Cardiac End of the Stomach by Surgical Extirpation. Richard H. Sweet, M.D., Boston, Mass.

The Use of the Roux Y in Extending the Operability of Carcinoma of the Stomach and of the Lower End of the Esophagus. John T. Reynolds, M.D., Chicago, Ill.; John P. Young, Jr., M.D., Chicago, Ill.

Surgical Treatment of Cancers of the Gastric Cardia. George T. Pack, M.D., New York; Gordon McNeer, M.D., New York.

The C. V. Mosby Company
3207 Washington Blvd.
St. Louis 3, Missouri

Acct 2-48

Please send me Pack's CANCER of the Esophagus and Gastric Cardia. The price is $5.00

☐ Enclosed find check. ☐ Charge my account.

Name_____

Address_____

(In writing to advertisers, please mention the Journal—it helps.)

BBL

Media for the Cultivation of Brucella

TRYPTICASE SOY BROTH

for *isolation,* especially from blood, and for use wherever a fluid medium is desirable.

TRYPTICASE SOY AGAR

for use with dyes for *identification* of species, and for plate and slant cultures.

CYSTINE TRYPTICASE AGAR

for *maintenance* of stock cultures.

Prices and literature sent on request

BALTIMORE BIOLOGICAL LABORATORY

1640 Gorsuch Avenue Baltimore-18, Md.

ALBIMI

BLOOD CULTURE MEDIA

Prepared in the

"McCARTNEY BOTTLE"

not only

IDEAL FOR BED-SIDE USE

but also

SOLVES MAILING PROBLEM

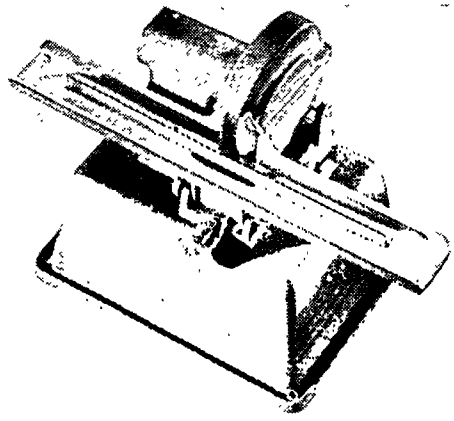
Leaflet on request

ALBIMI LABORATORIES

460 SEVENTH AVENUE
BROOKLYN 15, N. Y.

INDISPENSABLE

in your laboratory...



the Berkeley

MICROTOME KNIFE SHARPENER

for perfect edges...three times faster

Operation is simple, eliminates honing and stropping. Assures hollow-ground edges that cut better and last much longer. *Send for our Illustrated Bulletin MKS-14*

Berkeley Scientific Company

SIXTH AND NEVIN AVENUE • RICHMOND, CALIF.

SHILLABER'S

Non-Drying

IMMERSION OIL

for Microscopy

OFFERS THESE ADVANTAGES OVER CEDAR OIL..

- *Better Results
- *Safer
- *Time Saving
- *Neutral
- *Low Fluorescence
- *2 Viscosity Ranges
- *Controlled Optical Properties



PRICES (Grade A or Grade B)

COMBINATION PACKET	
1 Ounce A with 1 Ounce B	$1.00
1 Ounce Bottle Each	.50
4 Ounce Stock Bottle	1.50
16 Ounce Stock Bottle	4.00

ONE OUNCE BOTTLES HAVE APPLICATOR RODS

Order Through Your Laboratory Supply Dealer Or Direct From

R. P. Cargille 118 Liberty Street New York 6, N. Y.

Boyd—Text-book of Pathology

By WILLIAM BOYD, M.D., M.R.C.P., F.R.C.P.

Professor of Pathology and Bacteriology, University of Toronto, Canada

Fifth Edition

This is a complete pathology—physiologic in its outlook—covering, in condensed but comprehensive form, the essentials of both surgical and internal pathology. It is *pathology of the living patient*, presented vividly and accurately by this outstanding authority.

In revising this work for the enlarged *fifth edition*, Dr. Boyd has brought the entire book fully up-to-date. So much new material has been added that it was necessary to reset the entire book. In addition, a discussion of allergy, omitted from the previous edition, has been rewritten and reinserted into the text. But Dr. Boyd did more than just revise and bring up-to-date the subject matter. He has supplemented the text with 24 new black and white illustrations and an additional color plate, to make the entire work even more practical than before. There are now 500 black and white illustrations and 30 plates in color.

The *Journal of the Association of American Medical Colleges* spoke of this work as "*. . . a real introduction to medicine.*" It may well be called the most useful text-book of pathology which the student, intern or practitioner may purchase.

1049 Pages. 500 Illustrations and 30 Plates in Color. $10.00

Bell—Text-book of Pathology

By E. T. BELL, M.D.

Professor of Pathology, University of Minnesota. In contributions by the editor, B. J. Clawson, M.D., Professor of Pathology and J. S. McCartney, M.D., Associate Professor of Pathology, University of Minnesota, Minneapolis.

Sixth Edition

Clinical medicine is a direct continuation of pathological studies, not an abrupt entrance into a new field. For this reason, Dr. Bell designed his book to be both a text for medical students and a useful reference book for practicing physicians.

The work offers a complete review of the field of general pathology. Conclusions are painstaking and conservative and there is a sound approach to each concept not often obtained in more lengthy treatises. Subject matter is listed under well-defined headings and so grouped as to make the information easily available. All of the illustrations are original and the subject is presented as a liviug science that explores the nature and causes of disease on which all successful practice must ultimately be based. This edition retains the outstanding characteristics of its predecessors. It is brief, yet comprehensive, clear and definite, authoritatively written and well organized.

". . . a classical textbook. The subjects discussed are comprehensive enough to be intelligible, in a style lucid and most readable. The format is good. The paper and printing are most satisfying features. The index is thorough, the references . . . authoritative."—*New Orleans Medical and Surgical Journal.*

910 Pages. 500 Illustrations and 4 Plates in Color. $10.00

LEA & FEBIGER

Washingon Square
Philadelphia 6, Pa.

Please send me the books indicated below:

☐ Check enclosed ☐ Bill me at 30 days ☐ Charge on your partial payment plan

☐ Boyd—Text-book of Pathology.....$10.00

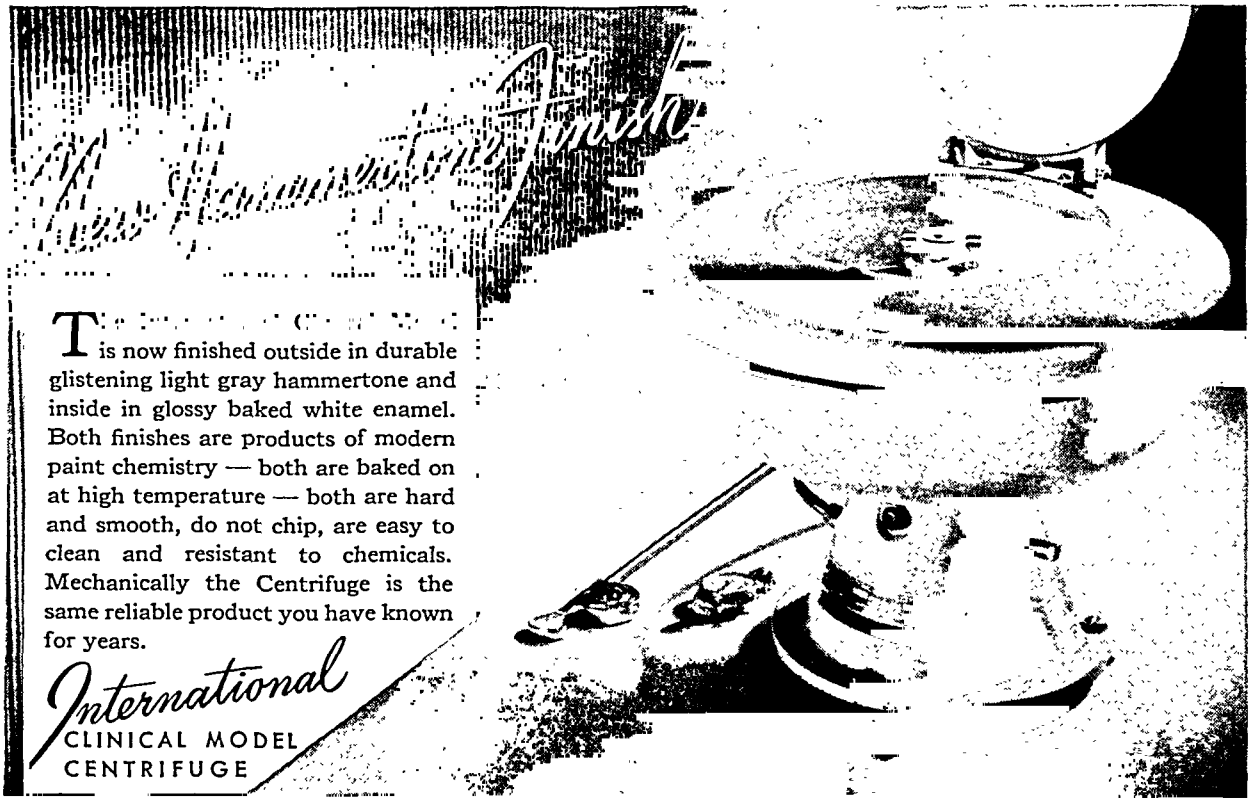
☐ Bell—Text-book of Pathology..... 10.00

DR. (please print)..... ADDRESS

CITY..... ZONE....STATE.....

(A.J. Cl. Path. 2-49)

(In writing to advertisers, please mention the Journal—it helps.)



INTERNATIONAL EQUIPMENT COMPANY *Boston 35, Mass.*

REICHERT MICROTOMES

It gives us great pleasure to announce that REICHERT MICROTOMES of Vienna are again available.

We are now in a position to accept orders for the following internationally recognized instruments:

REICHERT LARGE SLIDING TYPE MICROTOME "OmE"

REICHERT FREEZING TYPE MICROTOME "OmP"

Write now for full information to:

LABORATORY EQUIPMENT COMPANY

118 Eleventh Street — San Francisco 3, California

LABORATORY C&B REAGENTS

Inorganic and Organic Chemicals

•

Biological Stains Solutions

•

Chemical Indicators Test papers

•

Write for copy of our new catalog

The COLEMAN & Bell Co., Inc.,

Manufacturing Chemists: Norwood, O., U. S. A.

COLEMAN & BELL

(In writing to advertisers, please mention the Journal—it helps.)

AT HOME OR AWAY SPOT TESTS SIMPLIFY URINALYSIS

No Test Tubes • No Measuring • No Boiling

Diabetics welcome "Spot Tests", (ready to use dry reagents), because of the ease and simplicity in using. No test tubes, no boiling, no measuring; just a little powder, a little urine—color reaction occurs at once if sugar or acetone is present.

Galatest . . . Acetone Test (DENCO)

FOR DETECTION OF SUGAR IN THE URINE — FOR DETECTION OF ACETONE IN THE URINE

SAME SIMPLE TECHNIQUE FOR BOTH

1. A LITTLE POWDER

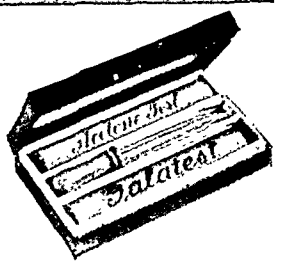


2. A LITTLE URINE

COLOR REACTION IMMEDIATELY

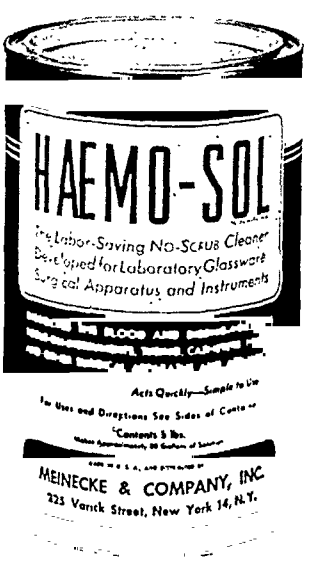
Accepted for advertising in the Journal of the A.M.A.

WRITE FOR DESCRIPTIVE LITERATURE



A carrying case containing one vial of Acetone Test (Denco), one vial of Galatest, medicine dropper and Galatest color chart is now available at all prescription pharmacies and surgical supply houses. This is very convenient for the medical bag or for the diabetic patient.

Acetone Test and Galatest — THE DENVER CHEMICAL MANUFACTURING COMPANY, INC. 163 Varick St., New York 13, N.Y.



Literature and samples on request. Write us regarding your specific cleaning problems.

NO RESIDUE

TO INTERFERE WITH YOUR EXACTING TITRATIONS AND TESTS

Good technique demands that laboratory glassware be not merely washed but *thoroughly cleansed.* This you can be sure of when you use HAEMO-SOL, the original chemically prepared cleaning agent and blood solvent, specifically developed for laboratory and hospital work.

Of equal importance, laboratory glassware must be free of even the slightest trace of detergent-induced deposit or residue. HAEMO-SOL affords you this protection due to its extraordinary solubility and free rinsing qualities, indicated by the fact that it dissolves to a crystal-clear solution evincing no turbidity or precipitate on standing. Furthermore, HAEMO-SOL'S apparent non-etching effect on glass, correlated with its low pH, saves replacement expense on costly laboratory apparatus.

MEINECKE & COMPANY, INC.

225 VARICK STREET • NEW YORK 14, N. Y.

(In writing to advertisers, please mention the Journal—it helps.)

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
STATE COUNSELORS 1948

Alabama	ALBERT E. CASEY, 1907 Wellington Road, Birmingham 9
Arizona	O. O. WILLIAMS, 2020 N. 11th Ave., Phoenix
Arkansas	M. J. KILBURY, 926 Donaghey Bldg., Little Rock
California	J. W. BUDD, 1407 S. Hope St., Los Angeles 15
	P. MICHAEL, 434 30th St., Oakland 9
Colorado	E. R. MUGRAGE, 4200 E. 9th Ave., Denver 7
Connecticut	R. E. KENDALL, 30 Lexington Rd., Hartford
Delaware	J. W. HOOKER, Memorial Hospital, Wilmington
District of Columbia	O. B. HUNTER, SR., 915 19th St., N.W., Washington 6
Florida	L. Y. DRYENFORTH, 1022 Park St., Jacksonville 4
Georgia	A. J. AYERS, Medical Arts Bldg., Atlanta 3
Idaho	J. A. BEEMAN, 309 Idaho St., Boise
Illinois	O. SAPHIR, Michael Reese Hospital, Chicago 16
Indiana	W. DODDS, Culver Hospital, Crawfordsville
Iowa	F. H. LAMB, 220 Main St., Davenport
Kansas	J. L. LATTIMORE, 618 Mills Bldg., Topeka
Kentucky	H. GORDON, 2543 Broadmeade Rd., Louisville
Louisiana	E. H. LAWSON, 2700 Napoleon Ave., New Orleans 15
Maine	J. E. PORTER, 22 Arsenal St., Portland
Maryland	C. W. STEWART, 6 E. Read St., Baltimore
Massachusetts	D. A. NICKERSON, Salem Hospital, Salem
Michigan	C. I. OWEN, Grace Hospital, 4160 John R., Detroit 1
	HAZEL PRENTICE, 458 W. South St., Kalamazoo 46
Minnesota	A. H. WELLS, St. Lukes Hospital, Duluth 5
Mississippi	R. H. FENSTERMACHER, 920 Crawford St., Vicksburg
Missouri	H. N. ALLEN, 816 Missouri Bldg., St. Louis 3
	V. B. BUHLER, General Hospital, Kansas City 8
Montana	R. F. PETERSON, Murray Clinic, Butte
Nebraska	J. P. TOLLMAN, 42nd St. and Dewey Ave., Omaha 5
Nevada	L. PARSONS, 235 W. 6th St., Reno
New Hampshire	J. B. HOLYOKE, Mary Hitchcock Memorial Hospital, Hanover
New Jersey	S. A. GOLDBERG, Presbyterian Hospital, Newark
New Mexico	T. R. MORAN, St. Joseph Hospital, Albuquerque
New York	A. A. EGGSTON, 653 Park Ave., New York 21
	V. W. BERGSTROM, 21 Park Ave., Binghamton
North Carolina	C. C. CARPENTER, Bowman-Gray School of Medicine, Winston-Salem 7
North Dakota	L. W. LARSON, Clinic Bldg., Bismarck
Ohio	F. BAYLESS, 10300 Carnegie Avenue, Cleveland 6
Oklahoma	W. F. KELLER, Medical Arts Bldg., 119 North Broadway, Oklahoma City 2
Oregon	V. D. SNEEDEN, U. of Oregon Mdeical School, Portland 1
Pennsylvania	C. J. BUCHER, Jefferson Hospital, Philadelphia 7
	T. R. HEMBOLD, R.D. 9, Box 229, Pittsburgh 16
Rhode Island	R. J. WILLIAMS, Rhode Island Hospital, Providence
South Carolina	K. M. LYNCH, Medical College of South Carolina, Charleston 16
South Dakota	R. L. FERGUSON, University of South Dakota, Medical School, Vermillion
Tennessee	A. C. GOSE, 1203 Eastmoreland Ave., Memphis 4
Texas	J. J. ANDUJAR, 2951 Bentbrook Blvd., Fort Worth 4
Utah	J. H. CARLQUIST, Dr. Groves Latter-Day Saints Hospital, 8th Ave. and C St., Salt Lake City
Vermont	E. H. BUTTLES, 457 S. Willard St., Burlington
Virginia	T. J. MORAN, The Memorial Hospital, Danville
Washington	C. P. LARSON, Tacoma General Hospital, Tacoma
West Virginia	F. C. HODGES, 800 First National Bank, Huntington
Wisconsin	S. B. PESSIN, St. Mary's Hospital, Milwaukee 11
Wyoming	S. S. ZUCKERMAN, 1606 Capital Ave., Cheyenne
Hawaii	I. L. TILDEN, The Clinic, 881 S. Hotel St., Honolulu 53, T. H.

(In writing to advertisers, please mention the Journal—it helps.)